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THE RELATIVE INCIDENCE OF INTESTINAL PARASITES IN HOSPITAL PATIENTS IN NASHVILLE AND IN RURAL TENNESSEE*

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RECENT interest in the intestinal protozoa of man in the United States has stimulated the publication of several reports of the incidence of these parasites in cities in various parts of the country. The reports of Williamson, Kaplan and Geiger from Chicago[1] and Andrews and Paulson from Baltimore[2] give the incidence of all of the protozoa found, while that of Faust[3] gives only the incidence of *Endamoeba histolytica*. The data for the intestinal protozoa and helminths for Nashville are given in the present report. If similar reports can be published from time to time from other cities a more accurate index of these parasites can ultimately be secured for the cities of the country as a whole.

The present report is a summary of the routine fecal examinations made in the laboratory of the Department of Preventive Medicine and Public Health of the Vanderbilt Medical School between Jan. 1, 1930, and June 30, 1932.† Of the 2,112 white and negro persons examined, 1,231 were adult medical out-patients and 881 were a miscellaneous group including pediatrics out-patients and in-patients, adult hospital patients suffering from gastro-intestinal complaints and a few similar outside patients of private physicians. All of the adult out-patients and most of the other group were persons who could not afford the attendance of a private physician. They came both from

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the city of Nashville and from the surrounding rural districts, and some from other cities. Many who resided in the city had previously lived on farms where they may have acquired their intestinal parasites. Because of this fact no attempt has been made to separate them into urban and rural groups. The results represent the incidence of these parasites from the combined urban and rural population of middle Tennessee.

The technic of fecal examination was as follows:

1. A heavy smear preparation in saline was examined for *Strongyloides* larvae and eggs of helminths.

2. A thin smear preparation in saline was examined for motile protozoa.

3. A salt-flotation test was performed for eggs of helminths.

4. About a cubic centimeter of the specimen was emulsified in tap water by shaking in a 25 c.c. test tube and filtered into a 50 c.c. centrifuge tube through "sheet-cotton" made absorbent by soaking in gasoline. The cotton was washed through with tap water up about 40 c.c., and the filtrate centrifuged at about 1,800 revolutions for one-half to one minute. The supernatant fluid was poured off and a portion of the sediment was examined in iodine eosin solution for protozoan cysts.

5. In cases where the identification of motile amebas or cysts was doubtful in fresh preparations, smears were stained with iron hematoxylin.

Most of the patients received only one fecal examination. When more than one was made it was usually done either to check the previous finding of *E. histolytica* or to follow the effect of treatment, or to make a more thorough search for the parasite in cases suspected of harboring it. This report may therefore be considered on the basis of one examination per person.

Table I shows the results of these examinations. It will be seen that *E. histolytica* was found in 63 patients or 3.0 per cent of the series. Of these 63 patients, only two were suffering from frank amebic dysentery. One other showed trophozoites which were identified as *E. histolytica* by stained

TABLE I

SUMMARY OF FECAL EXAMINATIONS ON 2,112 PATIENTS IN VANDERBILT HOSPITAL JANUARY 1, 1930 TO JUNE 30, 1932

	1,231 FROM MEDICAL O. P. D.		881 FROM OTHER SOURCES		TOTAL	
	NO. POS.	% POS.	NO. POS.	% POS.	NO. POS.	% POS.
One or more parasites	340	27.6	302	34.3	642	30.4
Endamoeba histolytica	38	3.1	25	2.8	63	3.0
Endamoeba coli	172	14.0	97	11.0	269	12.7
Endolimax nana	96	7.8	57	6.5	153	7.3
Iodamoeba bütschlii	44	3.6	24	2.7	68	3.2
Giardia lamblia	34	2.8	81	9.2	115	5.5
Chilomastix mesnili	42	3.4	31	3.5	73	3.5
Trichomonas hominis	16	1.3	19	2.2	35	1.7
Enteromonas hominis	1	0.1	5	0.6	6	0.3
Ascaris lumbricoides	3	0.2	50	5.7	53	2.5
Trichuris trichura	3	0.2	19	2.2	22	1.0
Hookworm	11	0.9	13	1.5	24	1.1
Hymenolepis nana	5	0.4	19	2.2	24	1.1
Strongyloides stercoralis	4	0.3	10	1.1	14	0.7
Enterobius vermicularis	0	0.0	3	0.3	3	0.14

smear and culture, and three others showed both trophozoites and cysts. Only cysts were found in the specimens from the other 57 patients. A few of these were suffering from gastrointestinal complaints but most of them came to the hospital for trouble not apparently associated with amebic infection.

The other protozoa found in this series appeared approximately in the incidence which would be expected if the incidence of *E. histolytica* in the group is taken as a basis for comparison. The incidence of the various helminths is also in accord with what is to be expected in a combined city and rural group in this geographic region. It might be noted that *Strongyloides* larvae were found in fourteen patients, which is more than half as frequently as hookworm eggs were encountered. This indicates that *Strongyloides* is of some importance as a parasite of man in this region even in a city hospital. It will also be noted that all of the helminths as well as *Giardia* had a higher incidence in the in-patient group than in the out-patients. This is probably to be accounted for by the fact that a considerable proportion of the latter group were children, in whom the incidence of both *Giardia* and the helminths is generally higher than in adults.

Table II compares the results of the Nashville summary for protozoa with those from Chicago, Baltimore, and New Orleans. It will be seen that Baltimore seems to enjoy the lowest incidence, Chicago comes second, Nashville third, and New Orleans highest. One would expect the incidence to be progressively higher from north to south and these studies suggest that it is, with the exception of Baltimore. The patients examined by Andrews and Paulson[2] in Baltimore were a selected group in that they all suffered from gastrointestinal complaints. It is conceivable that such patients sometimes may lose their intestinal protozoa because of an unfavorable environment produced by other pathologic conditions in the gastrointestinal tract. It would be interesting to know whether an unselected series of hospital in-patients or out-patients in Baltimore would show a higher incidence of intestinal protozoa. In the case of New Orleans, one would expect to find a higher incidence of intestinal protozoa there than in the inland cities of the South since New Orleans is exposed to the importation of such infections from the tropics.

A comparison of the incidence of the human intestinal parasites for Vanderbilt Hospital with that for the rural population of eight of the surrounding counties and for the rural population of the State of Tennessee as a whole is given in Table III. The data for the surrounding counties and the state are taken from the recent surveys reported by Meleney, Bishop and Leathers[4] for the protozoa and by Keller, Leathers and Bishop[5] for the helminths. This table shows that the incidence of almost every parasite is higher in the surrounding rural area than in the Vanderbilt Hospital patients. This can partly be explained for *Giardia* and for the helminths by the fact that most of the specimens in the rural survey came from school children, in whom the incidence of these parasites is generally higher than in adults. In the case of the amebas, however, this would not apply, since their incidence has been universally found to be higher in adults than in children. The lower incidence of the amebas in the Vanderbilt Hospital patients than in the surrounding

TABLE II

HOSPITAL SURVEYS IN THE UNITED STATES FOR INTESTINAL PROTOZOA

AUTHORS	YEAR	PLACE	TYPE OF PATIENTS	NUMBER OF CASES	PER CENT POSITIVE FOR										NUMBER OF SPECIMENS	METHOD OF EXAMINATION
					E. HISTOLYTICA	E. COLI	E. NANA	I. BÜTSHLII	D. FRAGILIS	GIARDIA	CHILOMASTIX	TRICHOMONAS	EMBADOMONAS	ENTEROMONAS		
Williams, Kaplan and Geiger	1929	Chicago, Ill.	Food Handlers	1,148	2.4	19.2	2.0	5.3	--	6.5	1.9	1.8	--	--	2	Fresh specimen straight smear
Williams Kaplan and Geiger	1929	Chicago, Ill.	Normal (?) Persons, Mostly Hospital Out-Patients	380	1.1	8.1	0 8	1.7	--	1.7	1 6	1.1	--	--	1-2	Fresh specimen straight smear and concentration
Andrews and Paulson	1931	Baltimore, Md.	Hospital Out-Patients with Gastrointestinal Complaints	522	0.2	4 2	2 5	1.3	--	2.7	1.3	2.1	0 2	--	1	Straight smear. Specimens brought to clinic
Faust	1931	New Orleans, La.	Male Medical Wards Female Medical Wards Obstetric Service Ambulatory Clinic Patients (Dr. F. M. Johns)	234	27.2 13.1 25.2 7.3										Two-thirds received 3 or more examinations	Straight smear
Meleney	1932	Nashville, Tenn.	Hospital Out-Patients and In-Patients	2,112	3.0	12.7	7.3	3.2	0.0	5.5	3.5	1.7	--	0.3	1	Smear and concentration Specimens brought to clinic or sent from wards

TABLE III

INCIDENCE OF HUMAN INTESTINAL PARASITES IN VANDERBILT HOSPITAL PATIENTS AND IN THE RURAL POPULATION OF TENNESSEE

	PROTOZOA						HELMINTHS			
	E. HISTOLYTICA	E. COLI	E. NANA	IODAMOEBA	GIARDIA	CHILOMASTIX	ASCARIS	TRICHURIS	HOOKWORM	HYMENOLEPIS NANA
Vanderbilt Hospital	3.0	12.7	7.3	3.2	5.5	3.5	2.5	1.0	1.1	1.1
Rural population of eight surrounding counties	9.5	32.0	13.3	4.3	15.0	3.5	16.7	2.5	0.2	1.8
Rural Tennessee as a whole	11.4	32.1	11.9	4.1	14.7	2.9	27.1	7.6	6.8	2.9

rural area is probably due to the fact that the majority of the hospital patients were residents of the city, where excreta disposal is more sanitary, where flies are somewhat less abundant, and where personal hygiene is in general better than in the rural areas.

Table III also shows that the counties surrounding Nashville have an incidence of intestinal protozoa approximately the same as that for the rural population of Tennessee as a whole. In the case of the helminths, however, these counties are considerably below the incidence for the whole state due to the fact that these counties are situated mainly in the "Central Basin" of the state, where the conditions necessary for the transmission of the helminths are less favorable than in some other parts of Tennessee.

It should be remembered that the incidence percentages here reported do not represent the actual incidence in the groups studied. It is estimated that one fecal examination by the technic employed for protozoa will reveal about half of those actually harboring *E. histolytica*. On this basis the actual incidence of this parasite in the Vanderbilt Hospital patients would be about 6 per cent, that in the surrounding rural area would be about 19 per cent and that for the rural population of Tennessee as a whole would be about 23 per cent.

The higher incidence of intestinal protozoa in the rural areas is emphasized particularly in order to focus attention upon the rural population in connection with the problem of amebiasis in the United States.

Andrews and Paulson[2] in the report of their Baltimore study state, "The incidence of intestinal protozoa in such a civilian" (apparently meaning "city") "group as has been described, is probably more nearly representative of the incidence of intestinal protozoa in residents of the temperate zone as a whole than incidences based upon the previously mentioned surveys" (institutions, troops, mining camps, remote rural districts) "in as much as most of the population of the temperate zone now is concentrated within large cities." This statement leaves the rural population too much out of consideration. The United States Census for 1930 shows that 43.8 per cent of the population of the whole country is rural, and that even in Maryland 40.2 per cent is rural. In the portion of the country designated in the Census as "The North" 32.8 per cent, or nearly one-third of the population, is still rural, and

in the portion designated in the Census as "The South," which includes Maryland, 65.9 per cent, or nearly two-thirds, is rural. It is evident, therefore, that the rural population must still be considered important in determining the incidence of intestinal protozoa in the United States.

Again Craig[6] in speaking of the amebiasis problem states, "It is undoubtedly true that the vast majority of infections with *Endamoeba histolytica* in this country are acquired through food handlers in public eating places" (institutions, hospitals, hotels, restaurants, etc.). "While in rural communities infection undoubtedly occurs in the home, and while this is also true to a lesser extent in the cities, it is no less true that the great source of infection in this country is the food handler in public eating places." Craig[6] thus focuses attention on towns and cities as the location of the main problem of amebiasis in the United States. It is undoubtedly true that public food handlers are an important source of infection in cities, and the necessity for their control should not be minimized. On the other hand, the data which have been presented for Tennessee in Table III show that the incidence of amebiasis in the rural areas is three or four times as high as in the city, and it is fair to assume that this does not apply to Tennessee alone. This indicates that the conditions for transmission of the dysentery ameba are much more favorable in the rural districts than in the cities. In the matter of sewage disposal and in the protection of excreta and food from flies, American cities present on the whole much better conditions than most rural districts. Food handlers in public eating places, although they may have no adequate conception of the hygienic handling of food, usually have some degree of supervision which would at least tend to limit the frequency with which they would convey the parasite to the patrons of these places. In the poor rural home, however, where close personal contact constantly occurs, often under the most unhygienic conditions, the opportunities for transmission within the family are much greater. The mother as food handler and nurse, the children constantly playing together, and the fly with its free access to feces and food probably all contribute to the spread of the intestinal protozoa in the family. The long duration of these infections is undoubtedly responsible for their continuation and spread in new family groups by marriage. Frequent visiting between families, and the roaming habits of the fly are probably responsible in the main for transmission from house to house. It seems important, therefore, to focus attention upon the amebiasis problem in rural districts as well as in cities. It must be remembered that the adult population of cities is made up to a significant degree of persons who spent their childhood in rural districts, and that such persons often are employed as domestic servants and public food handlers. It is therefore possible that sources of infection in cities may frequently have their origin in rural areas.

In conclusion it may be said that the results of the examinations in Vanderbilt Hospital indicate that the careful routine examination of fecal specimens from hospital patients anywhere in the United States is well worth the effort in revealing the occasional presence of pathogenic animal parasites, and in discovering a pathologic condition which might not otherwise be diagnosed.

It is undoubtedly true, as Craig[6] states, that fecal examinations are often more important for a correct diagnosis than some of the other diagnostic measures ordinarily employed as a routine at the present time.

SUMMARY

1. The results of routine fecal examinations for animal parasites in 2,112 hospital patients in Nashville, Tennessee, are reported. The incidence is somewhat higher for most of the intestinal protozoa than is reported for Chicago and Baltimore, but lower (for *E. histolytica*) than is reported for New Orleans.

2. The incidence of most of the intestinal parasites in hospital patients in Nashville is lower than for the surrounding rural area, and lower than for the rural population of Tennessee as a whole.

3. The problem of amebiasis in Tennessee is apparently more a rural problem than an urban problem, though the latter should not be ignored.

4. Careful routine fecal examinations of hospital patients should be employed much more than is the custom at the present time in the United States.

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STUDIES IN THE PHARMACOLOGY OF LOCAL ANESTHETICS

VI. Comparison of Metycaine With Cocaine and Procaine on Experimental Animals

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INTRODUCTION

PRELIMINARY pharmacologic data on certain of McElvain's[1] local anesthetics, piperidine derivatives, have been reported by McElvain and Jones[1] and Rose.[2] Some of these compounds were submitted to Dr. W. R. Meeker of Mobile, Alabama, for comparative clinical evaluation. Dr. Meeker[3] finally selected the compound gamma—(2-methyl-piperidino)—propyl benzoate hydrochloride as the compound which showed promise of a useful local anesthetic.

Since that selection considerable pharmacologic data have been reported on this compound by Coles and Rose[4]; Rose, Coles, and Thompson[5]; Coles and Rose[6]; and Rose.[7]

This article is a continuation of the pharmacologic study of this compound in comparison with cocaine and procaine, particularly, the action of gamma—(2-methyl-piperidino)—propyl benzoate hydrochloride (metycaine) on the perfused heart of frogs, on the blood pressure and respiration of anesthetized and pithed dogs, on the perfused legs of frogs (peripheral vessels), and on the sensory nerve trunk (sciatic of rabbits).

Action on the Perfused Frog Heart.—The hearts of large leopold frogs were used. The perfusion experiments were performed during the months of September and October. The Howell modification of Ringer's solution was used; that is, NaCl 0.7, $CaCl_2$ 0.026, HCl 0.03, the quantities referring to grams of the anhydrous salts per 100 c.c. of distilled water. This solution had a P_H of 7.2 to 7.3 (colorimetric). The temperature of the solution and of the hearts was kept at 22° C. to 24° C. during the experiments. The hearts were perfused essentially according to the method of Sollmann and Barlow[8] under constant pressure from the vena cava, the fluid escaping from the wounds and from the inferior end of the vena cava. The perfusion was made by a heart perfusion cannula (Dr. Greene's).

Various dilutions (1:5,000, 1:10,000, 1:20,000, 1:40,000, and 1:80,000) of the local anesthetics, cocaine, procaine, and metycaine, were used. At least 3 frog hearts were used for each dilution.

Cocaine with a dilution of 1:5,000, shows a very marked decrease in rate and amplitude and inhibition of rhythm. This is also observed with dilutions of 1:10,000, 1:20,000, and 1:40,000. A dilution of 1:80,000 produces no change in amplitude and rhythm and only a slight decrease in rate (see Fig. 1).

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Procaine, with a dilution of 1:5,000, shows a distinct change in rate and amplitude and an inhibition of rhythm, although not equal to that of cocaine (1:5,000). Dilutions of 1:10,000 and 1:20,000 of procaine produce a slight decrease in amplitude and in rate without disturbance of rhythm. Dilutions of 1:40,000 and 1:80,000 of procaine produce a distinct stimulation, and an increase in amplitude and rate (see Fig. 2).

Metycaine (1:5,000) produces an auricular ventricular block. Metycaine (1:10,000, 1:20,000, and 1:30,000) produces a decrease in amplitude and a decrease in rate without disturbance of rhythm (see Fig. 3). Dilutions of

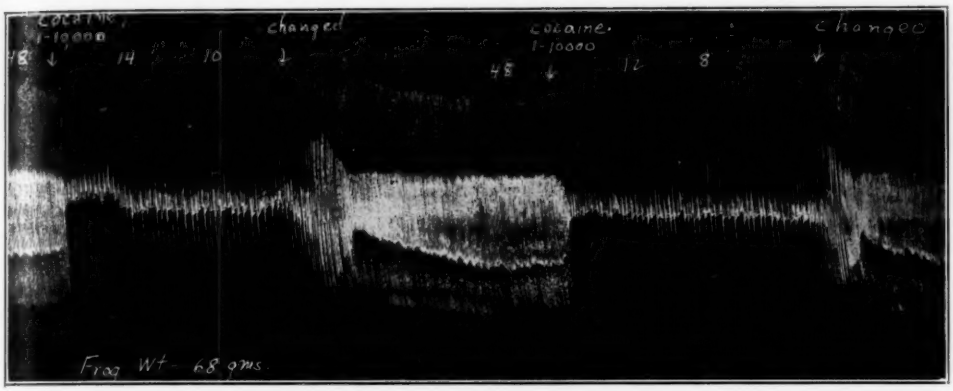

Fig. 1.—Represents a tracing of a perfused frog heart. The normal heart rate is 48. Following the perfusion of cocaine 1–10,000, the heart shows an A-V block with a marked change in rate.

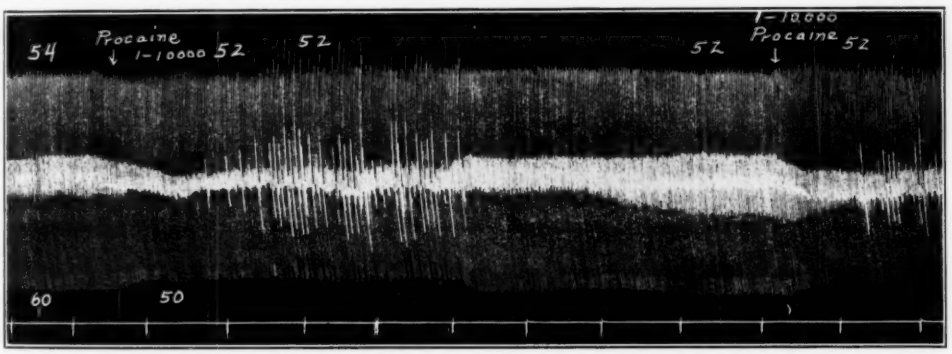

Fig. 2.—Represents a tracing of a perfused frog heart. The normal heart rate is 54. Following the perfusion of procaine 1–10,000, the heart rate is comparatively unchanged and there is only a slight decrease in amplitude.

1:40,000 and 1:80,000 produce a slight stimulation in 50 per cent of the hearts. Thus, metycaine produces less depressing symptoms on the perfused frog heart than cocaine but is more depressant than procaine.

Action on the Blood Pressure and Respiration of Anesthetized (Ether and Sodium Amytal) Dogs.—Fourteen dogs were used, 6 dogs under ether and 6 dogs under sodium amytal (40 mg. per kg. intravenously). Two dogs were pithed. The vagi were cut in some of the anesthetized experiments. Doses of 1 mg. to 10 mg. per kg. of cocaine, procaine, and metycaine were injected intravenously. In some of the experiments, alternating doses of the local anesthetics were injected in the same animal.

The effects of all three of the local anesthetics on the general circulation are partly central and partly peripheral. Cocaine in small doses (1 to 2 mg. per kg.) causes a distinct increase in blood pressure and a slower pulse rate. In moderate doses (3 to 5 mg. per kg.), the pulse is increased and a rise in blood pressure is observed. This rise in blood pressure and change in pulse rate, with small doses and moderate doses, is less in dogs with vagi cut, and practically absent in pithed dogs. Large doses (5 to 10 mg. per kg.) of cocaine produce in anesthetized and pithed dogs a very great fall of blood pressure and a weak, slow pulse.

Procaine in small doses (1 to 2 mg. per kg.) produces a very slight rise in blood pressure and very little change in pulse rate in anesthetized (ether and sodium amytal) dogs. In dogs with vagi cut or pithed, the rise is absent. The rise, which occurs after the use of small doses of cocaine, is generally higher than the rise produced by procaine. Procaine, in moderate doses (3 to 5 mg.

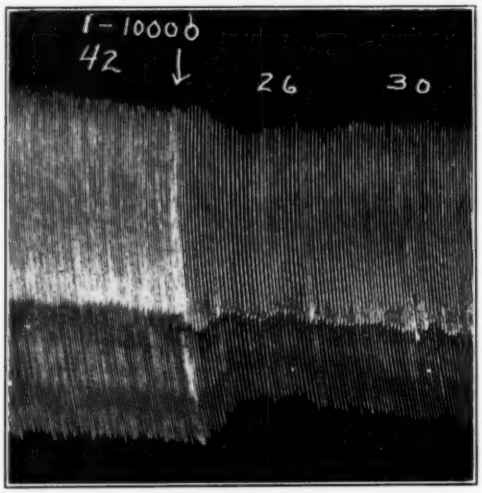

Fig. 3.—Represents a tracing of a perfused frog heart. The normal heart rate is 42. The arrow shows the change from control solution to a 1-10,000 metycaine solution. Numbers 26 and 30 represent the heart rate following the perfusion of the local anesthetic. There is a decrease in amplitude.

per kg.), generally causes no change or a drop in blood pressure and an increased pulse rate. Procaine in large doses (5 to 10 mg. per kg.) produces in experiments a distinct drop in blood pressure and a slow pulse; however, equivalent doses of cocaine produce a greater drop in blood pressure and a slower pulse.

Metycaine in small doses (1 to 2 mg. per kg.) produces either a very slight rise or no change in blood pressure, and very little change in pulse rate. With small doses (1 to 2 mg. per kg.), the fall of blood pressure predominates in anesthetized dogs (vagi cut) or pithed dogs. Metycaine in moderate doses (3 to 5 mg. per kg.) either produces no change or a slight fall in blood pressure, and a distinct increase in pulse rate. Metycaine in large doses (5 to 10 mg. per kg.) produces a fall in blood pressure and a slow, weak pulse rate. This fall of blood pressure and slow pulse rate with equivalent doses is more than with procaine and less than with cocaine.

The effects on respiration with small doses (1 to 2 mg. per kg.) of cocaine, metycaine, and procaine are acceleration, the degree of acceleration being in the order named. Moderate (3 to 5 mg. per kg.) doses of cocaine, metycaine, and procaine increase the respiration rate, but decrease the amplitude. Large doses (5 to 10 mg. per kg.) of cocaine, metycaine, and procaine cause depression of respiration, the degree of depression being in the order named.

Action on Perfused Frog Legs (Peripheral Vessels).—The perfusion method of frog legs used was essentially that of Trendelenburg's.[9] The frog was pithed, a cannula (Greene) inserted into the abdominal aorta. The outflow from the abdominal vein was recorded by the number of drops. The local anesthetics were perfused from a Mariotte bottle through a "Y" connection.

At least 2 or more frogs were used on each dilution. The dilutions used were 1:1,000, 1:2,500, and 1:5,000 of each local anesthetic. Epinephrine 1:40,000 dilution, and sodium nitrite 1:1,000 dilution were used to show vasoconstriction and vasodilatation after the local anesthetic was given. The results of these experiments are given in Table I.

TABLE I

PERFUSION OF FROG LEGS

(Laewen-Trendelenburg Method)*

DRUG	DILUTION	NUMBER OF FROGS	TIME OF MAXIMUM EFFECT	DECREASE (CONSTRICTION)	INCREASE (DILATATION)
			minutes	drops	drops
Cocaine	1: 1,000	3	15	4	–
Cocaine	1: 2,500	2	15	3	–
Cocaine	1: 5,000	3	15	–	4
Metycaine	1: 1,000	2	15	0	0
Metycaine	1: 2,500	3	15	0	0
Metycaine	1: 5,000	2	15	0	0
Procaine	1: 1,000	2	15	0	0
Procaine	1: 2,500	2	15	0	0
Procaine	1: 5,000	2	15	0	0
Sodium nitrite	1: 1,000	16	10-30	–	10
Epinephrine	1:40,000	16	10-20	35	–

*Laewen, A.: Arch. exper. Path. Pharm. **51**: 416, 1904. Trendelenburg, P.: ibid. **63**: 165, 1910.

As shown in Table I metycaine and procaine produce neither constriction nor dilatation. Cocaine produces constriction in dilutions of 1:1,000 and 1:2,500 and dilatation in dilutions of 1:5,000.

Action on the Sensory Nerve Trunks (Sciatic).—The method used is essentially that of Biberfeld and Pototsky and elaborated by Schmitz and Loevenhart.[10] In this study, the technic of the latter was used.

As shown in Table II, a 0.75 per cent solution of metycaine produces anesthesia equivalent to a 3 per cent solution of cocaine and a 0.5 per cent solution of procaine.

Action on Smooth Muscle.—The action of metycaine, cocaine, and procaine on smooth muscle was determined on the isolated intestinal strips of rabbits, isolated bladder of rats, and isolated uteri of guinea pigs and rabbits.

On the isolated intestinal strip of rabbits, the inhibition of normal rhythm is distinct with all three local anesthetics. Metycaine in dilutions of 1:80,000 produces inhibition equivalent to cocaine 1:30,000 and procaine 1:40,000.

TABLE II

MINIMUM EFFECTIVE CONCENTRATION FOR NERVE BLOCKING (RABBIT'S SCIATIC)

DRUG	4 PER CENT		3 PER CENT		2 PER CENT		1 PER CENT		0.75 PER CENT		0.5 PER CENT		0.25 PER CENT	
	NUMBER OF EXPERIMENTS	RESULTS	NUMBER OF EXPERIMENTS	RESULTS	NUMBER OF EXPERIMENTS	RESULTS	NUMBER OF EXPERIMENTS	RESULTS	NUMBER OF EXPERIMENTS	RESULTS	NUMBER OF EXPERIMENTS	RESULTS	NUMBER OF EXPERIMENTS	RESULTS
Cocaine	3	Complete anesthesia	3	Complete anesthesia	10	No anesthesia in 4, partial anesthesia in 3, complete anesthesia in 3	3	No anesthesia	3	No anesthesia	5	No anesthesia	5	No anesthesia
Procaine	3	Complete anesthesia	3	Complete anesthesia	3	Complete anesthesia	3	Complete anesthesia	3	Complete anesthesia	5	Complete anesthesia	5	Complete anesthesia in 1, partial anesthesia in 1, no anesthesia in 3
Metycaine	3	Complete anesthesia	3	Complete anesthesia	3	Complete anesthesia	3	Complete anesthesia	5	Complete anesthesia in 3, partial anesthesia in 2	5	Complete anesthesia in 2, partial anesthesia in 1, no anesthesia in 2	5	No anesthesia

The action of these compounds on the isolated bladder strips of rats shows contraction and a distinct increase in rhythm, the order of their increased effectiveness in dilutions of 1:5,000 and 1:10,000 being metycaine, cocaine and procaine.

In dilutions of 1:5,000 and 1:10,000, these local anesthetics produce a distinct contraction of the isolated uterus of guinea pigs and a contraction and increased rhythm of the isolated uterus of rabbits, their effectiveness in equivalent dilutions being in the same order, metycaine, cocaine, and procaine. Thus, the action of metycaine in equivalent dilutions on smooth muscle is apparently more effective than either cocaine or procaine.

CONCLUSIONS

1. Metycaine produces less depressing symptoms on the perfused frog heart than cocaine, but is more depressant than procaine.

2. The comparative effects on the general circulation and respiration of metycaine are more like procaine than cocaine.

3. Metycaine on the peripheral vessels is more like procaine than cocaine.

4. The action of metycaine on the sensory nerve trunk (sciatic) is two-thirds as effective as procaine and more than three times as effective as cocaine.

5. Metycaine on smooth muscle is more effective than either procaine or cocaine; that is, in equivalent doses, metycaine produces more inhibition of isolated intestinal strips and more stimulation of isolated uterine or bladder strips than either cocaine or procaine.

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A FATAL CASE OF SICKLE CELL ANEMIA WITH AUTOPSY FINDINGS*

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SICKLE cell anemia, an hemolytic anemia, in which many erythrocytes have a characteristic sickle or crescent shape, was first described by Herrich in 1910. Since that time the disease has been reported with increasing frequency by several observers, viz., Washburn, J. E. Cook, J. Meyers, V. R. Mason, V. P. Sydenstricker, J. G. Huch, and others.

It is a familial and hereditary disease confined largely, if not altogether, to the negro race. In those cases apparently white, investigation of the ancestry has usually suggested mixed blood. The condition is congenital, being present at birth, and is probably due to congenital defects in the spleen and blood-forming organs that result in changes in the erythrocytes which predispose them to hemolysis and phagocytosis. The anemia, when present, is due to excessive blood destruction activated, perhaps, by factors that in a normal person would be innocuous. It exists in a latent and active phase. The latent phase presents neither definite symptoms nor physical signs and is recognized only by special blood examination. The active phase presents definite symptoms and physical signs and the characteristic sickling may be seen on an ordinarily stained blood smear.

A number of cases have been reported in recent years, probably the largest series by Sydenstricker. In view of the fact that case reports with autopsy findings are very few, we feel justified in presenting this case with autopsy report.

CASE REPORT

Case 1.—Colored male, aged twenty-one, was admitted to the hospital on Nov. 27, 1931. At this time he complained of swelling of the entire body, shortness of breath, weakness and vertigo. The onset of the present illness was six months prior to admission, at which time he noticed edema of the feet and legs which gradually extended to the abdomen, face, and arms. Dizziness, weakness, dyspnea, palpitation, and headaches had been troublesome. Occasionally there was moderate epigastric pain brought on by drinking water or eating. On a previous admission to the hospital, September, 1931, a diagnosis of chronic nephritis was made. At this time he was anemic, red cell count 2,030,000, but the phenomenon of sickling was not noted. He remained in the hospital four weeks with subsidence of the edema and improvement of his general condition.

Past History.—He had gonorrhea in 1929 without complications, diphtheria in 1927, pneumonia in 1922, mumps and measles when a small child, frequent attacks of tonsillitis, and several ulcers on the anterior surfaces of both legs six or eight years ago. His mother died at the age of forty-one of Bright's disease. There was no history of blood dyscrasias in the family.

*From Shreveport Charity Hospital.
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Physical Examination.—He was a fairly well-developed and nourished colored male with generalized edema and moderate dyspnea. There was an icteric tinge to the sclerae and the tonsils were hypertrophied and cryptic. A few moist râles were heard over the bases of the lungs. There was a systolic murmur heard best at the apex of the heart, not transmitted, and there was no apparent enlargement of the organ. Blood pressure was 150 systolic and 70 diastolic. The abdomen contained free fluid and the liver was palpable two finger-breadths below the costal margin. The spleen was not palpable. There were several scars on the anterior surfaces of the legs about the middle third.

The patient remained in the hospital continuously from Nov. 27, 1931, to Feb. 2, 1932. Treatment was largely of a supportive nature. A suitable donor for blood transfusion could not be obtained either from his family or those who volunteered. Liver extract, ventriculin with iron and iron alone had no effect on the anemia. He ran a low grade temperature up to the last week, then 103.4° F. which we attributed to an exacerbation of the nephritis and bronchopneumonia. He died Feb. 2, 1932.

The report of the laboratory work, made at intervals during his stay in the hospital, is given in Table I.

Necropsy.—The body was that of a well-developed colored male, aged twenty-one, with generalized edema. The pupils were equal, round, and measured 5 mm. in diameter. There was slight jaundice of the sclerae and marked pallor of the conjunctivae. The teeth and gums were in good condition, tongue normal in appearance and consistence and the buccal mucosa was pale. The edema of the upper extremities was quite marked from the elbows downward, including the wrists and hands. The abdomen bulged. The pubic hair was normal in quantity and distribution. The thighs and legs were equal and the skin, which pitted on pressure, was tense and shiny. The posterior cervical and inguinal lymph nodes were palpable. There were several scars on the anterior surfaces of the legs.

Abdomen.—The subcutaneous adipose tissue had a maximum thickness of 5 cm. in the midline anteriorly and presented a pale, gelatinous appearance due to the accumulation of fluid which was given up freely on compression. Several liters of lemon colored, slightly turbid fluid were found in the peritoneal cavity. The peritoneum was smooth and was tinged yellow in color. The liver extended the breadth of four fingers below the costal margin in the midclavicular line, right. The appendix was small, retrocecal, and the serous covering was pale and smooth. There was marked edema of the mesentery and the fat in the omentum, which was rather scant, was a peculiar pale brownish color, and was water-logged. The mesenteric lymph nodes were enlarged and sectioned surfaces were pale pink. The *liver* weighed 2250 gm., edges were rounded, and the capsular surface was smooth. Externally it was bluish red in color. Sectioned surfaces bulged slightly and were generally red or brownish red with small patches of yellow here and there. Surfaces were bathed with thin, watery blood. The *spleen* was found as an elongate, wedge-shaped body lying along the gastrolineal ligament. Its inferior extremity was in contact with the tail of the pancreas while the superior pole did not quite extend to the stomach. Its position was well toward the median line and much lower than the position occupied by a normal organ. It measured 5 and 10 mm. respectively at the upper and lower extremities in width and its greatest thickness was 8 mm. It was 4 cm. long. There were deep wrinkles in the thick, gray capsule. Sectioned surfaces were brownish in color, dry, slightly retracted, and presented a sievelike appearance. The *pancreas* was normal in size, firm, and the lobules were conspicuous. Surfaces were pink in color. The *suprarenals* were normal in size, the pigmented zone normal in color and width, and the medullae were pinkish in color. The *kidneys* were about equal in size and weighed together 430 gm. There was considerable yellowish red fatty tissue about each organ. The capsules stripped easily exposing smooth, mottled surfaces with persistent fetal lobulations. There was slight increase in cutting resistance and surfaces bulged slightly. The cortices measured from 5 to 9 mm. in thickness and the striations were blurred and indistinct. The glomeruli were noted as minute, bulging red points. The pyramids were slightly swollen and, like the cortices and outer surfaces, mottled. The pelvic fat was not excessive and the pelvic lining was smooth and pale. The *ureters* were free from abnormalities, normal in size, and the lining was

TABLE I

LABORATORY DATA

URINE			
DATE	11/27/31	12/15/31	1/21/32
Specific gravity	1.013	1.010	1.010
Reaction	Alk.	Neut.	Acid
Albumin	4-plus	1-plus	2-plus
Sugar	None	None	None
Acetone	None	None	None
Casts	Occ. hyaline	None	Hy. F. & G. Gr.
Pus cells	Many	Few	Loaded
Epithelial cells	Bladder	Occ. sq.	Sq. & blad.
Crystals	Phos.	Phos.	
Amorphous sed.	Heavy	S.nall amt.	
Bacteria	Mod. No.	Few	Many
Erythrocytes	Many	None	Few

BLOOD			
DATE	11/30/31	12/15/31	2/2/32
Total white	13,350	14,509	17,950
Neutrophiles	58	59	76
Lymphocytes	40	28	17
Eosinophiles	2	5	
Basophiles		1	
Large mon.		7	7
Total R. B. C.	1,700,000	1,940,000	1,230,000
Hemoglobin	33%	30%	20%
Color index	0.9		
Platelets	260,100	224,100	
Coag. time	2 min.		
Bleed. time	6 min.		
Nucleated reds	Many	13	
Poikilocytosis	Marked	Marked	
Anisocytosis	Marked	Marked	
Achromia	Marked	Marked	
Polychromasia	Marked	Marked	
Sickling	Many		
Reticulocytes	6.5%		
Wassermann	Neg.		
Kahn	Neg.		
Total protein	4.05	6.75	
N. P. N.	33.3	26.4	
Cholesterol		298.4	
Albumin	2.06		
Globulin	1.90		
Urea		11.9	
Icteric index		30.3	
Fragility test	No hemol. in dilutions from 0.5 to 0.28		
Basal metab.	–13	–9	1/13 –7
Gastric analysis	Free HCl 13; Total acidity 51		

pale. The *urinary bladder* wall measured 1½ cm. in thickness and there were large, clear bullae involving the lining of the organ. On section, these latter were found to represent submucous accumulations of serous fluid. The *prostate* and prostatic urethra showed nothing noteworthy. The *stomach* was normal in size. The rugae were quite conspicuous, and there was diffuse bluish red or dark red color in the lining. There was edema of all parts of the wall of the small and large intestines as well as passive congestion of the lining.

Thorax.—Fluid of the same character as noted in the peritoneal cavity was present in both pleural cavities (amount estimated, 2 liters). The pericardial sac contained 750 c.c. of clear, straw-colored fluid. The *lungs* were small. They were dark red at the bases and posteriorly and were mottled gray and black at the apices and anteriorly. There were old interlobar and apical adhesions on the right. The cutting resistance was increased and sur-

faces were brownish red in color. Rusty, frothy fluid could be expressed from cut surfaces of both organs. There were no areas of consolidation and anthracosis was moderate but diffuse. The main bronchi contained frothy fluid and their lining was pale. The tracheobronchial lymph nodes were anthracotic. The *heart* weighed 410 gm. and was slightly ovoid in shape. There was a milk patch on the anterior surface of the right ventricle measuring 2 cm. in diameter. The epicardial fat was not excessive. There were no gross organic lesions of any of the orifices. The valve leaflets, cusps, and the mural endocardium were tinged

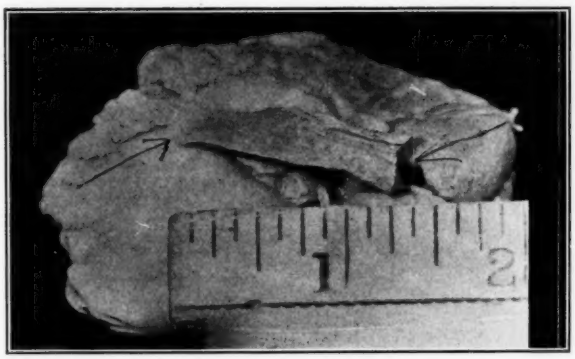

Fig. 1.—Spleen. Arrows point to the extremities of the organ. This represents full size except for ½ cm. removed for section. A portion of the pancreas is above and a section of the stomach wall below.

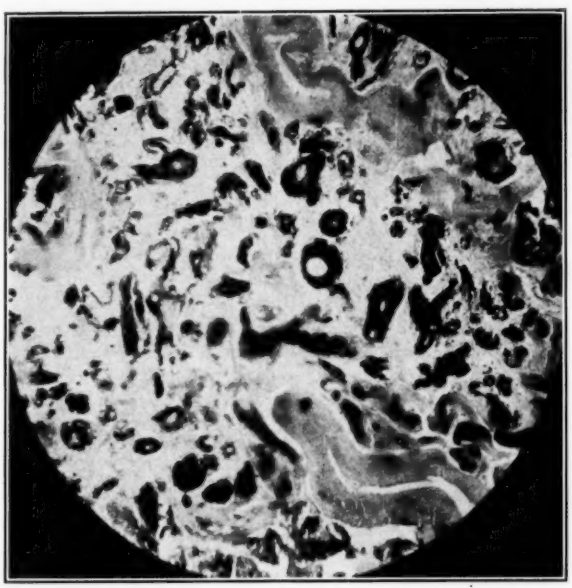

Fig. 2.—Section from spleen showing fibrosis of the pulp and necrotic malpighian bodies (dark areas). The thickened capsule is noted as infoldings (low power).

yellow. The myocardium was brownish red in color and fairly firm in consistence. The maximum thickness of the left ventricular wall at the base was 2½ cm. Rather conspicuous scars were noted in the left ventricular wall at its base. The lining of the coronary arteries and aorta was smooth and pale.

The convolutions of the brain were moderately flattened, and there was an excess of fluid in the subarachnoid space.

The bone marrow in the upper thirds of the tibiae and humeri were red.

Microscopic Examination.—Tissues were fixed in formaldehyde and paraffin sections stained with hematoxylin and eosin.

Heart.—There was moderate perivascular fibrosis which radiated between the adjacent muscle bundles. In addition, there were focal areas of degeneration with beginning connective tissue replacement. Relatively large areas of scarring which extended out to the epicardium were noted in some of the sections. There was diffuse engorgement of the small vessels. In places the nuclei were quite large.

Lungs.—There was an increase in the peribronchial and finer connective tissue about the alveoli. The alveolar septa were quite thick, due both to the feature mentioned above (fibrosis) and engorgement of the capillaries. Red cells were present in many of the alveoli as well as "heart failure cells." Many of the alveoli contained pink staining serous coagulum. The interstitial element was heavily laden in places with black pigment.

Aorta.—The aorta presented nothing noteworthy.

Spleen.—The capsule was thick and dense, and there were convolutions which extended deeply into the organ, almost meeting similar infoldings of the capsule from the opposite

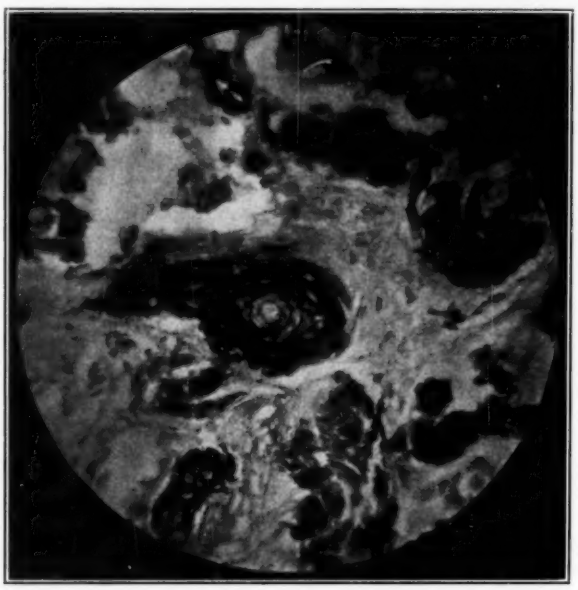

Fig. 3.—Section from spleen (high power).

side. Trabeculae were not identified. The pulp was completely replaced by dense, relatively acellular but somewhat loosely arranged connective tissue. This latter separated dark staining oval or oblong areas in which clear-cut cellular structure was conspicuously absent. These appeared to be composed of the necrotic remains of the malpighian corpuscles. In the centers of most of the dark staining areas was a less dense oval space containing either homogeneous pale staining material, old erythrocytes or dense connective tissue. Surrounding these was a darker zone of varying width composed of débris of necrotic cells and, in not a few instances, old erythrocytes and brown pigment.

Liver.—The capsule was slightly thickened. The central veins and adjacent portions of the sinusoids were engorged with erythrocytes, while the sinusoids at the periphery of the lobules showed this feature less conspicuously. The cell cords were relatively narrowed. There was diffuse hemachromatosis and cloudy swelling of the hepatic cells as well as fatty change in places. There was a varying amount of fibrosis, lymphocytic and, to a less extent, eosinophilic infiltration about the portal canals. In some instances fibrous strands radiated for some distance replacing the degenerated parenchyma. In such areas regeneration of bile capillaries was noted.

Kidneys.—The glomeruli showed a variety of changes which represented stages in the same progressive process. Proliferation of the subcapsular cells with formation of crescents or circles which in many instances showed fusion, fibrosis and hyalinization was a conspicuous feature. Many of the glomerular tufts were small and in some instances presented as small fibrous or hyalinized balls. Adhesion of the tuft of capillaries to the thickened capsule of Bowman was present in many places. Interstitial fibrosis and infiltration with lymphocytes, plasma cells, and an occasional eosinophile, particularly around the glomeruli showing advanced change, were widespread in the cortex. The tubules were dilated in many instances and contained a serous coagulum or hyaline casts. The tubular epithelium showed cloudy swelling, fatty degeneration and necrosis, not diffuse. The capillaries were generally engorged and the glomeruli, normal in appearance, completely filled the subcapsular space in most instances.

Pancreas.—Other than diffuse congestion and cloudy swelling, this organ showed nothing noteworthy.

Suprarenals.—Postmortem change.

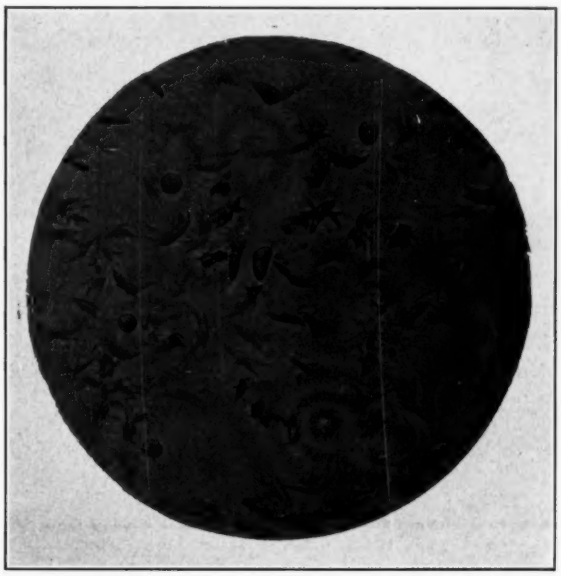

Fig. 4.—Wet preparation of blood showing sickling.

Lymph nodes.—The mesenteric and inguinal nodes showed diffuse fibrosis. There was an accumulation of large, pale macrophages as well as an occasional eosinophile and neutrophile in some of the sinuses. A few eosinophiles were present diffusely in the cortex and pulp cords.

Summary of Gross and Microscopic Diagnoses.—Chronic passive congestion, edema and anthracosis of lungs; chronic pleuritis, right; chronic interstitial pneumonia; chronic fibrous myocarditis with moderate hypertrophy of heart; milk patch on anterior surface, right ventricle; cloudy swelling, hemachromatosis, fatty change, passive hyperemia and slight perilobular fibrosis of liver; necrosis and atrophy of spleen; passive hyperemia and cloudy swelling of pancreas; subacute and chronic productive capsular glomerulonephritis; chronic lymphadenitis; hypertrophy of bone marrow; chronic hypertrophic gastritis; anasarca; hydrothorax, bilateral; hydropericardium; ascites; jaundice; anemia.

DISCUSSION

This patient presented the characteristic findings of sickle cell anemia with an associated nephritis. The blood examinations showed the characteristic anemia, sickling, and leucocytosis. In addition, a decrease in the fragility of the erythrocytes was noted, no hemolysis occurring in dilutions which began with 0.5 and ended with 0.28. In five latent cases, detected by routine examination of wet preparations on 137 negro patients, delayed fragility was also noted, hemolysis beginning in dilution 0.38 and incomplete in dilution 0.28.

The characteristic pathology appears to be confined to the spleen and bone marrow. The findings in neither have been uniform which, perhaps, is best interpreted by assuming that different stages of a progressive process were encountered. There may be enlargement or atrophy of the spleen. Congenital malformation of the sinuses with free escape of blood into the pulp and the formation of blood pools around the malpighian bodies are probably characteristic of the latent phase and the early or intermediate stages of the active form. Marked atrophy of the organ with complete fibrosis of the pulp and necrosis of the few remaining malpighian bodies, as in our case, probably indicate long duration of active disease.

The bone marrow picture is that of chronic infection or injury with compensatory hyperplasia. Here again, the findings have not been constant. In our case there was simple hyperplasia of the marrow, while in Graham's case, there was chronic productive inflammation with centrifugal involvement of the bone. Chronic osteomyelitis, periostitis, leg ulcers, and even osteitis have been observed radiologically and at necropsy, but there may be fatal cases with atrophy of the spleen and the terminal complications such as nephritis and pneumonia which show none of these.

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BLOOD CATALASE IN HEART DISEASE*

1. Preliminary Report

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IN 1863 Schoenbein[1] described the catalytic effect of some substance in vegetable and animal tissues which liberated oxygen from a solution of hydrogen dioxide. The activity of this principle could be diminished by the addition of weak acids, heat, and other anticatalytic agents and because of its evident biologic nature he considered it to be an enzyme and called it catalase. From close observation of the type of reaction it has been claimed[2] that this enzyme in tissues is relatively inactive but that in the first phase of its reaction with the dioxide it becomes an active catalase. Furthermore, its activity is in direct proportion to the hydrogen ion concentration of the solution, rising up to a certain point and in inverse proportion to the temperature within certain limits, becoming less active as the temperature rises. The optimum temperature for the reaction is about 10° C., but temperatures up to 40° C. cause little, if any, inactivation whereas at 58° C. about 25 per cent catalytic action is lost.[4, 5, 6, 7]

In testing for catalase different grades and brands of commercial hydrogen dioxide solution have given varying yields of oxygen with the same tissue but the errors could be eliminated by neutralizing the solution with sodium hydroxide.[8] Margolis and his coworkers[3] found that high concentrations of hydrogen dioxide destroyed catalase in tissues and drew attention to the necessity of proper dilution.

The function of tissue catalase has been a subject of considerable dispute and its true value is still unappreciated. The majority of authors feel and have shown by more or less conclusive deductions that it is an important metabolic factor, particularly in intracellular oxidative processes. Burge[9] found that the catalase content of tissues is dependent on or proportionate to the metabolism and varies with the respiratory exchange. A high protein diet, as one would expect from its specific dynamic action, has been shown to cause an increase in catalase content,[10, 11] and after alcohol ingestion a similar rise has been observed.[14] Experimentally a fall in tissue catalase has been produced by starvation,[12] castration, thyroidectomy,[13] and to a less extent by pancreatectomy.

Clinically, changes in catalase content have been recorded in many diseases. In primary anemia, a rise has been observed whereas in cachectic diseases,[15] cancer, hemolytic anemias, febrile diseases and pulmonary tuberculosis,[16] there is a distinct fall. Its association with the metabolic rate has been noted further in many experimental and clinical observations as in the re-

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ported associated variations of both in the same individual during different parts of the day,[17] the occurrence of less tissue catalase in young kittens than adult cats,[18] and the 70 per cent increase in the blood during a mountain sojourn as compared with an increase of 38 per cent in the erythrocytes.[19] Varying favorably with the differences in metabolic rates, Alexeeff, et al.,[20] have found that the catalase content of male blood exceeds that of the female by 10 per cent. Robben,[21] however, challenges this metabolism theory of catalase function and shows that catalase added to muscle exercised no effect on the respiratory exchange. Moreover, Stehle[8] found that catalase content of blood is directly proportionate to the concentration of red corpuscles, and is not related to biologic oxidative processes. Furthermore, in a large number of experiments on protein feeding, he failed to produce an increase in catalase except in one case. In order of quantity catalase has been found and measured in the following tissues: blood, kidney, liver, spleen, lungs, heart, brain, and muscles.[22] Blood serum contains no catalase,[23] corpuscles suspended in isotonic solution fail to give the typical reaction with hydrogen dioxide, but laked erythrocytes liberate oxygen from the reagent,[24] the intensity of reaction varying with the number present.[25]

Catalase determinations have been reported in human organs both in health and disease. Diseased kidney has a decreased value, the content being less with the more extensive pathology.[26, 27] The rôle of erythrocytes is emphasized in the study of a pneumonia lung. During the stage of red hepatization, the catalase activity is increased over that of a normal lung and in a hemorrhagic infarct it is enormously increased. On the other hand, during gray hepatization, the pulmonary catalase is decreased. During ether or chloroform anesthesia, the catalase content of all tissues is decreased,[28] asphyxia having the same effect.[26] There is no appreciable change in diabetes, senility, eclampsia,[23] and there is no relation between change of heart rate and blood catalase.[29] Sollmann[30] investigated the catalase activity of the oral mucous membrane and found that there was no diagnostic change in the various forms of sore throat.

In this study, the authors make no attempt to explain the rôle of blood catalase, but wish to point out the changes in heart disease and their diagnostic value.

TECHNIC

In our work, several methods were tried, many being complicated. The set-up which proved to be the simplest to operate and the most efficient is that described by Hawk and Bergheim[31] with one minor modification by us. Most of the pathologic cases were seen at the Cardiac Clinic of the Boston City Hospital between ten and eleven o'clock in the morning, and all the blood specimens were taken at that hour in the order of the patient's appearance at the clinic and without knowing the diagnosis. Each patient was questioned about the content of his breakfast; those having had meat or eggs were tested on the following day when protein was eliminated from the meal. Complete blood examinations were done when indicated. After the oral temperature and pulse rate were recorded, blood was drawn into a special calibrated white

blood corpuscle diluting pipette up to the five mark from a puncture wound in the ear lobe. The pipette was then filled with distilled water and the contents transferred to a small test tube, several distilled water washings of the pipette being added to the laked blood. This was then corked and stored with the other morning's samples in the laboratory at 16° C. for twenty-four hours.

To 85 c.c. of a 3.5 per cent solution of hydrogen dioxide,[32] 15 c.c. N/10 sodium hydroxide solution were added, both reagents having been stored at the same temperature as the blood. The above mixture was made up each day the tests were done and prepared afresh for each ten blood samples.

The diluted laked blood was introduced into a 250 c.c. distilling flask and 10 c.c. of neutral hydrogen peroxide solution added from a Johnson modified burette, the flask being rotated until a reaction became apparent, and the oxygen liberated collected over water by displacement in a graduated cylinder. After an arbitrary time limit of five minutes, from the moment the hydrogen dioxide solution was introduced, the number of cubic centimeters oxygen liberated was read directly from the cylinder.

One hundred thirty-six blood samples from the same number of apparently healthy individuals were examined in this way and the normal range was found to be 10 to 15 c.c. oxygen. One-fifth of that number and several of the pathologic cases listed in the table were checked on different days, the figures agreeing within 5 per cent.

Four hundred and twenty-nine cases were studied and the amounts of oxygen liberated by the individual bloods carefully checked. When a blood caused a 19 c.c. yield, it was repeated to be certain that it was not a borderline case and its proper classification ascertained. The average normal figure was found to be about 15 c.c., thus putting all the normal cases in the 10-20 group.

In the last two groups of Table I, we find practically all the cases of rheumatic and scarlatinal endocarditis. There were 116 cases of mitral and aortic valvular disease of rheumatic etiology in the third group, a few of which were decompensated. All these were felt to be inactive affections as determined by the general clinical picture of each case and observation over a period varying from weeks to years. Seven others in this third group were found to be active, some at first examination and others after close study, the average yield being 29 c.c. in these. In the final group 18 cases of active rheumatic endocarditis gave the typical high result, two having been thought to be subacute bacterial endocarditis but the diagnosis was never finally established.

The three septicopyemia cases fall into the first two groups and are sharply differentiated from the rheumatic endocarditis of the latter groups.

Thirteen cases of scarlet fever are listed in the lower range, all having no organic heart complications. Six other cases, known to have a heart lesion arising as a complication of the fever fall into the class of rheumatic endocarditis, two being definitely active. The results in these cases are of particular interest when we consider the newer theory of relation between rheumatic fever, chorea, tonsillitis, and scarlatina. In 1899 McCollum[33] reported three cases of endocarditis as a complication of scarlet fever in a series of 1000 consecutive cases. Broadbent[34] reported endocarditis in 0.58 per cent of scarlet

TABLE I

DISEASES AND NUMBER OF CASES STUDIED, CLASSIFIED ACCORDING TO OXYGEN YIELD

	0-10	11-20	21-30	OVER 30
Normal		135		
Aortitis, syphilitic	1	3		
Aneurism			1	
Myxedema		3		
Hyperthyroidism		2		
Colloid goiter		3		
Diabetes mellitus		8		
Infectious arthritis		24	1	
Arthritis deformans		14		
Leucemia, myelogenous	2			
Leucemia, lymphatic	1			
Epilepsy			1	
Psychoneurosis	7			
Nephrolithiasis	1			
Hodgkins disease	2			
Bronchopneumonia			1	
Angina pectoris		2		
Hypertension		16		
Nonvalvular heart disease	1	22		
Functional murmurs	7	11		
Diphtheria myocarditis	2	4		
Scarlet fever				
(a) Normal hearts	1	12		
(b) With active endocarditis			4	2
Rheumatic heart disease				
(a) Decompensated	1			
(b) Compensated and decompensated			116	
(c) Active			7	18
Gonococcal acute endocarditis		1		
Staphylococcal acute endocarditis	2			
Total cases 429				

fever complications while Reinhard[35] found it in only one case of his series. Nobecourt[36] believes that the same etiologic agent in scarlet fever, rheumatic fever, chorea, and tonsillitis cause similar heart lesions, and Fahr,[37] from his pathologic studies, affirms his opinion of the general similarity of heart affections in scarlet fever and rheumatic infection. Sutherland,[38] on the other hand, believes that an attack of scarlet fever sensitizes the endocardium in individuals susceptible to rheumatic infection and that such infection is latent in those cases becoming active in the presence of scarlatina. Swift[39] has classified the heart complications of scarlet fever as follows:

1. Toxic, similar to those complicating diphtheria and occurring in the first week of the disease. They are due to the action of the disease. They are due to the action of the toxin on the myocardium.

2. Septicopyemic, malignant and rare.

3. Allergic, which occurs in the third and fourth week of the disease and is due to an acquired hypersensitiveness to the streptococcus or its toxin.

Despite the fact that the number of cases is small, the blood reaction in scarlatinal endocarditis closely resembles that of rheumatic etiology and further study is warranted.

In five instances the catalase content of blood proved to be of no diagnostic value. The first case was that of a decompensated mitral stenosis in which the yield was only 10 c.c. The second, an epileptic, yielded 26 c.c., an

infectious arthritis 21 c.c., aortic aneurysm 24.5 c.c., and a bronchopneumonia 38 c.c. The last case was that of a prolonged migratory bronchopneumonia in which there were no heart complications. These determinations were repeated with identical results.

In the early part of our work, we attempted to correlate our catalase values with the valve affected, intensity of physical signs, degree of dyspnea and other symptoms, grade of hypertrophy, but so far have found no relation to these factors.

At the present, our knowledge consists in the recognition of normal catalase values in human blood from 0-20 c.c. A value of 21-30 indicates valvular disease, the final diagnosis being dependent on the entire clinical and laboratory picture. At about 26, the lesion is probably active and over 30 certainly active. This was illustrated in the case of a medical student, twenty-six years of age, who first came under observation in October, 1931. At that time he was examined routinely on admission to the school and a tachycardia (rate 110) noted. He complained of nervousness but otherwise felt well. Ten years before he had severe pains in his legs and was obliged to stay in bed for two weeks. From that time until his admission to medical school he had frequent sore throats but no return of joint pains and he had been frequently examined in school and college. Aside from the rapid pulse and cryptic tonsils the examination was normal. A month later a basal metabolism rate was advised because of the nervousness and persistent tachycardia. Two tests were performed, both being normal.

The patient was seen by one of the authors in December, 1931, and early signs of heart disease were noted. The pulse rate was 96, rhythm regular, heart not enlarged to percussion, but there was a short blowing systolic murmur at the apex, intensified by exercise and transmitted to the axilla. The first sound at the apex was short and of a snapping character and the second sound in the pulmonic area was reduplicated. A blood catalase test was performed, the result being 44. He was advised to enter a hospital but refused and was not seen again until Jan. 20, 1932. Then he complained of weakness, pallor, palpitation, and precordial pain. Examination of the heart revealed a long crescendo presystolic murmur and snapping first sound at the mitral area. The pulmonic second sound was greatly accentuated. Apical and radial pulse rates were 126. An electrocardiogram showed a P-R interval of 0.20 second and a QRS complex of 0.08 second duration. The blood catalase finding was 41. The patient was hospitalized and given large doses of salicylates together with complete rest. An orthodiagram revealed no heart enlargement.

The blood catalase quantity dropped to 28 c.c. oxygen yield over a period of two weeks and the pulse rate to 86. Tonsillectomy was performed on the fifteenth day and the patient discharged to his home on the sixteenth day after entry. At that time P-R interval was 0.16 second and the duration of the QRS complex 0.06 second.

The authors were unable to get in touch with the patient again until March 20, 1932, at which time he was obviously an ambulatory cardiac. He complained of intermittent attacks of fever, palpitation, dyspnea, precordial

pain and weakness. Physical examination of his heart revealed a rate of 100, enlargement to percussion, a long harsh diastolic murmur and short first sound at the mitral area.

The results of a blood catalase test were 40.5 c.c. and this dropped to 30 c.c. after a week's rest at his home and in May, 1932, he was seen again with the same symptoms and signs and a catalase value over 40 c.c.

In this particular case the authors feel that an earlier diagnosis might have been made by a catalase test when his tachycardia was first observed. The fact that a relatively short period of bed rest caused symptomatic and actual improvement as shown by the change in catalase content of his blood should suggest that a longer treatment would have arrested the lesion and probably brought the catalase value below 26.

The authors were fortunate to obtain permission to study the blood reaction and use the record of two patients who came to necropsy.

CASE 1.—This twenty-three-year-old white female entered the Boston City Hospital Jan. 12, 1931, complaining of easy fatigue ability, shortness of breath and swelling of the ankles of several years' duration. There was a history of rheumatic fever several years before and she had had a crop of red, tender nodules on her legs two months before admission, which persisted for five weeks. The patient was kept in bed for three weeks, during which time the temperature was normal and she was sent home with a diagnosis of rheumatic heart disease. On March 3, 1931, she was readmitted to the hospital. Fever was the only complaint at this time and had persisted since February 21, when she had a mild sore throat, chilly sensations, and pains and swelling of the wrists and hands. Examination revealed a fairly well-developed and nourished, young female, not acutely ill. The eyes, ears, nose, and throat were normal. According to the record, physical examination of the heart revealed that the point of maximum intensity was in the fifth interspace outside of the midclavicular line. There was a loud systolic murmur at the apex which was transmitted to the axilla; the first sound at the mitral area was loud and snapping and the second sound at the pulmonic area accentuated. The blood pressure was 110/70. The lungs and abdomen were normal, and there was no swelling of the joints. Examination of the nervous system revealed no abnormalities.

Laboratory Examinations.—Urine and sputums were normal. The white count was 15,600 on admission. Chest plates showed a mass to the right of the superior mediastinum, and an enlarged heart with a mitral deformity. Repeated blood cultures were negative except for one in which there was a growth of *Streptococcus viridans.* Late in her illness, the authors determined the catalase content of her blood and found it to be 18.5. This result, in view of the above studies, was not consistent with a diagnosis of subacute bacterial endocarditis. The catalase reactions repeatedly remained normal.

Two weeks after admission, the chart showed a wild, swinging temperature ranging from 97° to 104°, until demise Jan. 19, 1932. Many roentgenograms of the chest were taken, were interpreted variously, and did not aid in clarifying the situation. Except for small, red, tender, skin nodules cultures from which showed *Staphylococcus aureus* which kept recurring and the development of enlarged swollen and tender knees, the condition of the patient did not change much over a period of several months. Death occurred suddenly.

Clinical Diagnosis.—Subacute bacterial endocarditis, acute dilatation.

Postmortem Examination.—The body was that of a poorly developed, poorly nourished white female. There was marked swelling of the first phalanx of the middle finger on each hand. Both knee joints and the right ankle joint were markedly enlarged. Over the anterior surface of the right thigh were several round, white scars about 1.5 cm. in diameter.

Pleural Cavities.—The left pleural cavity was negative. The right pleural cavity in the region of the lower lobe was obliterated. The pleuras were thickened and on cross-section varied in thickness from 8 mm. to 2 cm. Patches of soft, yellow material, 0.3 to 1 cm.

in diameter were scattered through the dense gray tissue representing the pleuras. In the region of the eighth rib at its point of juncture with the vertebral column, situated behind the pleura was a pocket of thick, yellow fluid 4 cm. by 2 cm. The ends of the ribs in this region were eroded.

Pericardial Cavity.—Negative.

Heart.—Weight 410 gm. There was a moderate degree of enlargement. The muscularis was dark red and firm with no gross areas of scarring or softening. The endocardium was smooth and *all valves were negative.* Both coronary arteries were patent throughout. There were no congenital anomalies of the heart or of the pulmonary artery or aorta.

Spleen.—Weight 220 gm. This organ was enlarged. The capsule was thin and wrinkled. Section showed a dark reddish purple parenchyma of soft consistence. The cut surface scraped with ease.

Joints.—On opening the right knee joint, a quantity of thick, gray fluid containing many yellowish gray flakes, was discharged. There was about 200 c.c. of this fluid. The synovial surfaces were fibrin coated. The synovia was soft, red, and thickened. It was closely adherent to the cartilage over the medial condyle of the femur. The semilunar cartilages could not be identified. The joint cartilage was smooth and glistening, except over the area of attachment to the synovia.

ANATOMICAL DIAGNOSES

Old pleurisy with abscess formation.
Retropleural abscess.
Purulent arthritis.
Cardiac hypertrophy.
Chronic passive congestion of the liver.
Enlargement of the kidneys.

In this case the low blood catalase coefficient was the only finding inconsistent with a clinically typical picture of subacute bacterial endocarditis, and the only antimortem finding consistent with the absence of an endocarditis at autopsy.

CASE 2.—J. G., a forty-seven-year-old male restauranteur was admitted to the Boston City Hospital in November, 1931, complaining of febrile attacks of five months' duration. Symptoms of fever, weakness, and loss of weight came on after a tonsillectomy in June, 1931. He had lost about sixty pounds of his weight during this time. Physical examination was entirely negative aside from the intermittent fever. Roentgenograms of chest, gastro-intestinal system and skeleton were normal. The Kahn, Widal, agglutination tests for tularemia, red corpuscle count, and hemoglobin tests were normal. Blood cultures and repeated searches for blood and intestinal parasites were negative. There was a persistent leucopenia, the count varying from 2,700 to 4,400. The differential count was normal. Blood catalase determinations averaged 16 thus excluding the possible diagnosis of rheumatic heart disease or subacute bacterial endocarditis. Quinine by mouth and arsphenamine intravenously were finally tried empirically. The patient improved and was discharged during a remission in January, 1932.

The fever recurred and he was admitted to another hospital for two weeks where no diagnosis was made. He reentered the Boston City Hospital in March, 1932, complaining of fever for three weeks, vomiting and diarrhea following an injection of arsphenamine. Physical examination at this time revealed further loss of weight, an enlarged liver and spleen and areas of brownish pigmentation on chest and back. He had convulsions on the third day which became more frequent and severe. The patient died on the fourth day after reentry.

Clinical Diagnosis.—Unexplained fever; ? subacute bacterial endocarditis.

Anatomical Diagnosis.—Lymphoblastoma involving liver, spleen, bronchial and hypergastric nodes. Focal necrosis in liver.

SUMMARY

We offer herewith:

1. Some considerations relative to the catalase reaction.

2. Our technic and standardization.

3. Results in 429 cases.

4. Suggestions as to the relationship of scarlet fever endocarditis to rheumatic heart disease.

5. In a small series (6 cases) of diphtheria with definite heart damage, the catalase was normal.

6. Three specific cases: (a) Early diagnosis and course of activity by the catalase test. (b) Case clinically resembling rheumatic heart disease with subacute bacterial endocarditis (including one positive blood culture, *Streptococcus viridans*). Catalase was normal. (c) Doubtful case clinically. Negative evidence by means of test.

CONCLUSIONS

1. The determination of blood catalase is of value in making a diagnosis of rheumatic heart disease especially in doubtful cases.

2. It is of help in determining the activity or inactivity of a valve lesion.

3. Study of the blood catalase in active rheumatic endocarditis will aid in determining the length of time required for treatment.

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A STUDY OF BLOOD CALCIUM AFTER THE ADMINISTRATION OF SODIUM OXALATE TO NORMAL AND THYROPARATHYROIDECTOMIZED CATS*

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SINCE oxalate forms an insoluble compound with calcium, a number of investigators were led to study its effects on calcium in the body, and, although their results were obtained in experiments on different animals and by different methods, they all agreed that the administration of soluble salts of oxalic acid was accompanied by deprivation of calcium from the tissues. According to Sarvonat and Roubier,[1] a considerable loss occurred in the soft parts as well as in the skeleton when oxalate was given to guinea pigs, in sufficient amounts, for several days. Luithlen[2] reported the elimination of large quantities of calcium in the urine of rabbits after the administration of oxalate, and Gross[3] found that the calcium content of dog's blood, after subcutaneous injections, was reduced 50 per cent, while Vines[4] obtained similar results in experiments on rabbits after the administration of ammonium oxalate. McCrea[5] reported that blood calcium was likewise decreased in cats that received sodium oxalate, and Gley and Kokas,[6] in studies on rabbits and cats, and more recently Chiao Tsai and Fong Yen Hsu,[7] in experiments on dogs, furnished additional data that oxalate lowered the calcium level in the blood. With the exception of Vines and the Chinese investigators, no attempt has, as far as we know, been made to determine the speed of recovery, nor have any observations been reported on the effect of the amount, the frequency or the method of, administration of oxalate on the concentration of blood calcium. As we found that a relation existed between calcium depletion, produced by oxalate, and pharmacologic reactions,[8] a study of its action, with special reference to those factors, was considered particularly advisable. The present investigation was, therefore, undertaken and observations were made on the changes in blood calcium produced after the subcutaneous or intravenous administration of different amounts of sodium oxalate, attention being directed also to the duration of the effect and to the influence of smaller doses when given for a period of several days. Owing to the rôle which the parathyroids play in calcium metabolism, experiments were also carried out on the effects on blood calcium produced by oxalate after their removal, for the results under these conditions might throw some light on those obtained when it is given to normal animals.

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Methods.—Cats, which were kept on a uniform diet of salmon, bread and milk with water ad libidum, were the subjects of the experiments. As indicated above, sodium oxalate was given subcutaneously and also intravenously, in which case urethane anesthesia was sometimes employed. The doses administered by intravenous injections were 10 mg. sodium oxalate per kilo about ten minutes apart, and the amounts administered subcutaneously were 20 mg. per kilo every twenty or thirty minutes. Blood calcium was determined by the Kramer Tisdall[9] method, the blood for analysis being usually drawn from the heart, but it was sometimes obtained from the femoral artery, instead. In all cases, calcium was determined before as well as after oxalate, and in thyroparathyroidectomized animals analyses for calcium were made before the operation and also about ten to thirty minutes before the injection of oxalate was begun. In some experiments more than one analysis were made in the interval between extirpation of the gland and the administration of oxalate. The operations, for the removal of the thyroid and the parathyroid, were carried out under proper aseptic precautions, with ether as the anesthetic. Oxalate was given intravenously, usually within two days, but a longer interval was sometimes allowed to elapse.

Results.—Although depression of the calcium level of the blood by oxalate, was produced by subcutaneous as well as by intravenous injections, the effect, caused by the two methods of administration, varied considerably. One hour after the subcutaneous injection of a dose of 20 mg. sodium oxalate per kilo,

TABLE I

SERUM CALCIUM, AFTER SUBCUTANEOUS INJECTIONS OF SODIUM OXALATE

CAT	SEX	WT. KILO.	BEFORE OXALATE	AFTER 20 MG. OXALATE PER KILO DAILY						
			MG. CA PER 100 C.C. SERUM	MG. CA PER 100 C.C. SERUM, AMT. DECREASE, TIME AFTER INJECTION						
				INJECTION NO.						
				I	II	III	IV	V	VI	VII
7	female	2.3	9.8	10 2% 1 hr.	9.3 6% 1 hr.	8.6 13% 1¼ hr.	8.0 19% 25½ hr	—	—	—
8	female	2.4	9.7	9.7 0% 1½ hr.	7.9 19% 1 hr.	7.3 25% 1¼ hr.	—	—	—	—
10	female	2.3	10.0	9.6 5% 1 hr.	9.3 8% 1½ hr.	9.2 9 1 hr.	8.6 15% 1 hr. 5% 24 hr.	8.8 21% 1 hr.	—	10.0 1% 30 hr.
11	female	2.6	10.8	9.9 8% 1 hr.	9.9 8% 1½ hr.	9.1 16% 1 hr.	9.1 16% 1 hr. 11% 24 hr.	8.5 21% 1 hr.	10.3 4% 30½ hr.	—
12	female	2.6	10.5	10.4 1% 1 hr.	9.7 7% 1½ hr.	9.7 7% 1 hr.	9.5 9% 1 hr. 6% 24½ hr.	8.6 18% 1 hr.	10.4 0-1% 30½ hr.	—

practically no effect on blood calcium was observed in three out of five experiments, while the remaining two showed a decline of 5 and 8 per cent (Table I). The results were quite different when this dose was repeated for several days. While very marked changes in the concentration of calcium after the second dose were seldom observed, the fall being 6 to 8 per cent in 4 experiments and 19 per cent in only 1, the decrease of calcium in the blood was much greater after subsequent injections. The maximum diminution was 16 and 25 per cent after the third, 15 to 19 per cent when the fourth dose was given, 18 and 21 per cent after the fifth. We also observed that the depletion was progressive, the decrease of calcium reaching a maximum one hour after the third, fourth, and fifth injections, in different experiments, and, in one case, this was noticed even twenty-five and one-half hours after the fourth dose. Illustrative of the cumulative effect of oxalate was one experiment, in which no change was observed after the first dose, but the drop in the concentration of calcium was 19 per cent after the second, and 25 per cent after the third injection, and another, in which the first dose was likewise without demonstrable disturbance of blood calcium, the decrease was 6, 13, and 19 per cent after successive injections. Replacement apparently occurred as well as loss, as about twenty-four hours after the injection the calcium content of the blood was appreciably greater than it was one hour after the same amount was given, and in 3 experiments the effect of oxalate on blood calcium was negligible at the end of thirty hours (see Table I).

When the amount of oxalate was increased to 40 mg. per kilo, which was given subcutaneously in divided doses, and the blood was drawn one-half to one hour later, the fall of serum calcium was 2.5, 5.1, and 13 per cent in 3 experiments, but, in 2 others, it was 25 and 26 per cent. The results were not greatly different, in some experiments, with 80 mg. oxalate per kilo, as the decrease of calcium, half an hour after the injection, was 11 to 20 per cent in 4 experiments, but in 2 it was 36 and 37 per cent. The calcium content of the blood, four hours after the administration of these amounts of oxalate, failed to show any change from that previously observed, in some experiments. A distinct improvement occurred, however, in others, calcium showing a rise of 8 to 13 per cent above the previous level. On the other hand, a still further depression sometimes occurred, for in one experiment, with 40 mg. oxalate per kilo, the decrease of calcium was 25 per cent one hour after the injection and 32 per cent three and one-half hours later. Analysis of the blood within twenty-three to twenty-nine hours, after the administration of 40 mg. oxalate per kilo, indicated that the blood calcium regained its normal level in some cases and was 12 and 16 per cent below in 2 others. The changes were even more irregular after 80 mg. oxalate per kilo, the decrease of blood calcium in 2 experiments being greater on the day following than it was a few hours after the injection, and in one of these, depression of the calcium level continued as the concentration, fifty hours after oxalate was given, was less than on the previous day. In 4 other experiments, in which the fall of blood calcium half an hour after the administration of this amount of oxalate was 16 to 20 per cent, no significant change occurred in the course of about twenty-

four hours. But in 2 experiments in which, as mentioned above, pronounced depression of the calcium level was produced, the decrease amounting to 36 and 37 per cent half an hour after the injection of oxalate, the diminution of blood calcium was only 6 and 9 per cent about twenty-one hours later. To sum up the results obtained in this series of experiments, it may be stated that complete recovery was observed in about twenty-four hours only when 40 mg. oxalate per kilo were given, while in experiments with double this amount, the normal level was not restored until a period of forty-five to fifty-one hours after the injections was allowed to elapse.

TABLE II*

REPLACEMENT OF SERUM CALCIUM AFTER SUBCUTANEOUS INJECTIONS OF SODIUM OXALATE

CAT	SEX	WT. KILO	MG. CA PER 100 C.C. SERUM BEFORE OXALATE	OXALATE MG. PER KILO.	MG. CA PER 100 C.C. SERUM, AMT. DECREASE, TIME AFTER OXALATE			
4	female	2.2	10.7	40	9.3 13% 1 hr.	9.3 5.6% 4 hr.	11.0 slight increase 23 hr.	—
6	male	3.5	10.2	40	7.5 26% 1 hr.	8.4 17% 4 hr.	8.9 12% 24 hr.	—
9	female	3.7	9.8	40	7.4 25% 1 hr.	6.7 32% 4 hr. 20 min.	8.2 16% 29 hr. 45 min.	—
14	female	2.2	10.8	80	8.6 20% ½ hr.	8.8 19% 4 hr.	9.3 14% 27 hr.	—
3	female	3.0	10.6	80	6.7 37% ½ hr.	7.5 29% 5 hr.	9.6 9% 21 hr.	10.6 0% 45 hr.
5	female	2.1	10.1	80	6.5 36% ½ hr.	7.8 23% 4 hr.	9.5 6% 22 hr.	—
1	male	2.2	11.3	80	10.0 11% ½ hr.	9.1 19.4% 6¼ hr.	8.8 22.1% 23¼ hr.	7.8 30.9% 50½ hr.
2	female	2.75	11.5	80	9.3 20% ½ hr.	9.4 18.2% 6½ hr.	9.6 16.5% 23½ hr.	11.3 1.7% 50¼ hr.

*Tables II, IV, and V contain only some of the experiments given under the respective headings.

Wide variations in the decrease of blood calcium also occurred after intravenous injections even when the same amounts of oxalate were given. Six to ten minutes after the injection of 20 mg. sodium oxalate per kilo, the diminution of blood calcium was 10 to 28 per cent in 4 experiments, and, exceptionally, these differences were greatly increased, for while we found that, in one experiment, the fall, after one hour, amounted to 3.6 only, and the concentration of serum calcium in another was decreased 6 per cent, in a third it was 43 per cent. It may be remarked, in this connection, that the blood cal-

cium, before oxalate, was almost constant, being 10.0 to 10.3 mg. per 100 c.c. serum, while in one cat only it was 9.6 mg. Illustrative protocols, in brief, are given in Table III.

TABLE III

SERUM CALCIUM AFTER INTRAVENOUS INJECTIONS OF 20 MG. SODIUM OXALATE PER KILO

TIME	TREATMENT	MG. Ca PER 100 C.C. SERUM	PER CENT DECREASES OF CA	REMARKS
Cat 6, Male, Wt. 2.8 Kilo				
11:03 A.M.	11 c.c. blood drawn	10.3	18.0%	15 min. after second injection of oxalate
11:16 A.M.	2.8 c.c. 1% $Na_2C_2O_4$			
11:16 A.M.	2.8 c.c. 1% $Na_2C_2O_4$			
11:31 A.M.	12 c.c. blood drawn	8.3		
Cat 16, Wt. 3.8 Kilo				
11:53 A.M.	10 c.c. blood drawn	9.6	12.1%	6 min. after second injection of oxalate
11:57 A.M.	3.8 c.c. 1% $Na_2C_2O_4$			
12:10 P.M.	3.8 c.c. 1% $Na_2C_2O_4$			
12:16 P.M.	11 c.c. blood drawn	8.4		
Cat 62, Female, Wt. 2.0 Kilo				
10:30 A.M.	10 c.c. blood drawn	10.1	28.0%	10 min. after second injection of oxalate
10:40 A.M.	2 c.c. 1% $Na_2C_2O_4$			
10:50 A.M.	2 c.c. 1% $Na_2C_2O_4$			
11:00 A.M.	10 c.c. blood drawn immediately after death	7.3		

This inequality in the reduction of blood calcium, produced by the same amount of oxalate, is not easy to explain but, according to Percival and Stewart[10] and to Liu,[11, 12] the quantity of parathyroid hormone in the circulation determines the amount of diffusible calcium, and since oxalate combines with this fraction of the total calcium, the difference in the extent of depletion may thus be accounted for. A wide divergence in the effect produced was also observed when larger doses of oxalate were injected, for, ten minutes after the administration of 30 mg. per kilo, calcium was decreased 10 to 25 per cent in 7 experiments and, as in the case of the preceding experiments, the two extremes were met with, a reduction of 3 per cent only in one and 48 per cent in another. When the dose was raised to 40 mg. per kilo, the range was 25 to 49 per cent in 10 experiments, while in one case the reduction was only 12 per cent. Notwithstanding, however, the fluctuations in the calcium level observed after oxalate in these experiments, it was, nevertheless, found that the average decrease rose with the size of the dose.

In the absence of the complicating factors of the absorption of oxalate, the replacement of calcium in these experiments could be studied with greater advantage than after subcutaneous injections. There was scarcely any indication of recovery for about one and one-half hours after it was administered, as examination of the blood obtained at the end of this time, after 30 and 40 mg. per kilo, failed to show any appreciable change in the concentration of calcium from that observed when blood was drawn a few minutes after the intravenous injection of oxalate. On the contrary, a further decrease of calcium could sometimes be noticed since we found, in one experiment (5R Table IV), that the concentration fell 15 per cent below normal after ten minutes and 22 per cent fifty minutes later.

TABLE IV

SERUM CALCIUM AFTER INTRAVENOUS INJECTIONS OF SODIUM OXALATE INTO NORMAL CATS

CAT	SEX	WT. KILO	MG. CA PER 100 C.C SERUM, AMT. DECREASE					SOD. OXALATE, MG. PER KILO
			BEFORE OXALATE	TIME AFTER OXALATE				
				3-10 MIN.	1-1½ HR.	4 HR.	24 HR.	
55	female	3.0	10.3	8.4 19%	8.2 21%	—	9.9 5%	30
1R	—	2.9	9.9	8.4 20%	—	9.1 13%	—	
4R	female	3.7	10.1	9.2 11%	9.0 13%	9.7 6%	10.3 —	
5R	male	3.1	10.4	8.8 15%	8.1 22%	9.3 10%	9.4 9%	
13	—	—	—	6.6	6.6	7.3 10.6%	—	40
5A	male	3.5	10.4	6.4 38%	7.1 31%	7.7 26%	—	
61	female	2.2	11.0	4.2 62%	—	—	—	80
57	female	3.1	10.6	2.2 79%	—	—	—	120

A distinct tendency to recovery was indicated, however, by the results of the analysis of the blood obtained four hours after oxalate was injected. The decrease of blood calcium at this time, after 30 mg. oxalate per kilo, was only 6 to 13 per cent, the average being 8.4 per cent, whereas the reduction, ten minutes after this dose was given, varied between 11 to 25 per cent with an average of 18 per cent. In experiments with 40 mg. oxalate per kilo, the reduction of blood calcium at the end of two and one-half hours to five and one-half hours varied approximately between 21 and 27 per cent. With the exception of one experiment, in which the decrease at the end of five hours and forty minutes amounted to 8 per cent only, the average was about 25 per cent, representing a gain of 6 per cent over that observed six to seventeen minutes after the injection. The increase was thus 3 to 4 per cent greater after 30 mg. oxalate per kilo, during a period of about three to three and one-half hours. A further, and much greater, rise in the concentration of calcium occurred during the following period, of about twenty hours. In 2 experiments with 30 mg. oxalate per kilo, the blood calcium was only 5 and 6 per cent below normal, while in a third, restoration was complete. That the recovery was distinctly slower after 40 mg. oxalate per kilo was also shown in this case, as in one experiment the level of blood calcium was still 14 per cent below that of the control period.

When larger doses of oxalate were given intravenously, the depression of the calcium level was, of course, more pronounced than in the experiments with smaller amounts, but the relative decrease was not always proportional to the quantity injected. The loss was 8.1 mg. in one experiment one minute after the injection of 70 mg. oxalate per kilo, in another this was only 6.8 mg., though the dose of oxalate was 80 mg. per kilo, the percentile fall amounting to 66 and 62 per cent respectively. The loss was not greatly different when the dose of oxalate was still further increased. Three and five minutes after the injection of 110 and 120 mg. oxalate per kilo, the loss of calcium was 7.5

and 8.4 mg., the percentile reduction amounting to 73 and 79 respectively. As calcium depletion was 18, 31, and 66 per cent, after doses of 30, 40, and 70 mg. respectively, it shows that the depression increased with, but was out of proportion to, the size of the dose. This does not hold, however, in the case of still larger doses, for the average diminution of calcium after 110 and 120 mg. oxalate per kilo was about 76 per cent, which is considerably below the figure demanded if the decrease were proportional to these amounts. There is apparently a maximum dose of oxalate above which the decrease of calcium proceeds very slowly.

The blood calcium when oxalate was given after removal of the thyroid and parathyroids presented quite a different picture from that observed after it was administered to normal animals. The difference may be readily appreciated by comparing Tables IV and V, and is further emphasized in Table VI, in which the average losses of calcium in normal and in thyroparathyroidectomized cats are reported.

TABLE V

SERUM CALCIUM AFTER INTRAVENOUS INJECTIONS OF SODIUM OXALATE INTO THYROPARATHYROIDECTOMIZED CATS

CAT	SEX	WT. KILO	MG. CA PER 100 C.C. SERUM, AMT. DECREASE					OXALATE MG. PER KILO
			NORMAL	AFTER THYROPARATHYROIDECTOMY				
				BEFORE OXALATE	10 MIN. AFTER OXALATE	1 HR. AFTER OXALATE	4 HR. AFTER OXALATE	23 HR. AFTER OXALATE	
10	male	3.4	10.3	7.5 27%	6.6 12%	6.9 8%	6.2 17%	6.6 12%	30
7	female	2.9	11.1	7.5 32%	6.5 13%	6.6 12%	6.0 20%	7.2 17%	
6	male	2.8	9.4	5.2 45%	4.5 14%	4.6 14%	3.4 35%	4.1 2%	40
19	female	2.8	—	5.8	3.6 40%	—	—	—	80
22	female	3.0	10.6	6.3 41%	2.0 67%	—	—	—	140

As shown in Table VI, the absolute as well as the relative decrease of calcium was considerably greater in normal animals than after removal of the thyroid and parathyroids. It will be noticed that, about ten minutes after the injection of 30 mg. sodium oxalate per kilo into normal cats, the absolute de-

TABLE VI

REDUCTION OF SERUM CALCIUM AFTER OXALATE. EXPERIMENTS ON NORMAL (N) AND THYROPARATHYROIDECTOMIZED (T) CATS

OXALATE PER KILO	AVERAGE DECREASE OF CALCIUM, MG.	PER CENT DECREASE OF CALCIUM	REMARKS
N 30	2.0	18	Calcium determined in blood obtained about ten minutes after the intravenous injection of oxalate
T 30	0.45	13	
N 40	3.5	33	
T 40	0.8	17.3	
N 70-120	7.7	70	
T 80-140	3.5	60	

crease of calcium averaged 2.0 mg., while the relative decrease was 18 per cent. After the same amount of oxalate per kilo was given to thyroparathyroidectomized cats, the average decline was only 0.45 mg. calcium per 100 c.c. with an average of 13 per cent for the relative decrease. The fall of the calcium level was much greater when 40 mg. oxalate per kilo were given, the average decrease in normal and thyroparathyroidectomized animals being respectively 3.5 and 0.8 mg., while the relative decrease was 33 per cent in one and 17.3 per cent in the other. It may be remarked in this connection that these data do not include abnormal deviations from the above levels. The decrease of calcium after removal of the thyroids and parathyroids, in one experiment with 30 mg. oxalate per kilo, was negligible, while in another, after 40 mg. oxalate per kilo, the loss was 2.5 mg., whereas in the rest this varied between 0.7 and 1.0 mg. The loss of calcium was also much greater in normal than in thyroparathyroidectomized animals when the doses were considerably increased. After 70 to 120 mg. oxalate per kilo, injected into normal cats, the average decrease was 7.7 mg. calcium per 100 c.c. serum, and the relative decrease was 70 per cent. In thyroparathyroidectomized animals, that received 80 to 140 mg. oxalate per kilo, the absolute reduction averaged 3.5 mg. and the percentile fall was 60 per cent. Of interest also are the data presented above as showing that the difference in the decrease of calcium in normal and parathyroidectomized animals is much greater after small than after large doses, the ratios being about 4:1 and 2:1 respectively.

The duration of the effect of oxalate was also studied in cats after removal of the thyroid and parathyroids, but the observations were confined to experiments with doses of 30 and 40 mg. of sodium oxalate per kilo. The level of blood calcium, about one hour after the injection, was not greatly different from that observed ten minutes after the salt was given. In one experiment only was the difference appreciable as the decrease was 13 per cent ten minutes after oxalate and 23 per cent one hour later. The decline in the concentration of calcium at the end of four hours showed that it was only 8 per cent more in one and 9 per cent more in another, while in a third the decrease was, on the contrary, 4 per cent less than it was three hours previously. As the diminution, during the same period in another experiment amounted to 21 per cent, the earlier attempt at recovery may be regarded as exceptional. Although improvement occurred on the following day, the amounts of calcium were 0.9 to 1.3 mg. below those before oxalate, while the relative differences were 12 to 21 per cent. When the effect is compared with that produced by the same amounts of oxalate in normal cats, it becomes evident that the recovery is retarded after extirpation of the parathyroids.

DISCUSSION

The picture of the blood calcium in normal cats, before the intravenous administration of oxalate, presented a striking contrast to that observed after its injection. Whereas the concentration of calcium was practically constant or showed small differences only, before treatment, a wide range of variation occurred after oxalate, even when the same amount, in proportion to body

weight, had been administered. As the changes in blood calcium, when oxalate was given after removal of the parathyroids, manifested, on the contrary, only small variations, it is evident that a close relationship existed between parathyroid function and the extent of calcium depletion produced by oxalate in normal animals. The observations of Percival and Stewart as well as those of Liu, referred to above, have shown that the amount of diffusible calcium varied with the quantity of parathyroid hormone, while Trendelenburg and Goebel[13] and later Salvesen and Linder[14] found that it was the diffusible rather than the inactive calcium which was decreased in tetania parathyreopriva, which would explain the difference we observed, since it is this fraction which is, in all probability, acted upon by oxalate. As the hormone would be greatly reduced after extirpation of the parathyroids, a correspondingly lower decrease in calcium, produced by oxalate, should occur, as was indeed the case. This would be contradicted, however, by the large reduction of calcium, after doses of 80 to 140 mg. per kilo, which, as shown in Table VI, averaged 3.5 mg. Of interest in this connection is the suggestion of Salvesen and Linder[14] that the nondiffusible is transformed into diffusible calcium, in order to maintain the equilibrium between the two forms. That the diffusible calcium determined the extent of reduction, even after large amounts of oxalate were injected, was made highly probable by the greater decrease in normal than in thyroparathyroidectomized animals.

That the changes in blood calcium after subcutaneous injections of oxalate are also related to parathyroid function is suggested by the following observations. In experiments with small doses given daily, over a period of several days, it was found that the first injection was practically without any effect. Since the same amount given intravenously produced a considerable fall of blood calcium, the difference must be due to absorption. As oxalate when given subcutaneously would enter the circulation gradually, the small quantity of blood calcium thus bound is probably replaced by the activity of the parathyroid hormone, and the normal level is thus maintained. The reduction of calcium, when the injection of the same amounts of oxalate was continued for several days, may be explained if the toxicity and cumulative effect of the latter are taken into consideration. It has been shown by Salant and Swanson,[15] Gates and Meltzer,[16] Gates,[17] and later by Gross,[3] that oxalate is of itself toxic, and Salant and Swanson pointed out, in experiments on rabbits, that its effect was also cumulative, which was corroborated by us in experiments on cats. It is highly probable, therefore, that oxalate is injurious to the parathyroids, and that their function is depressed, thus interfering with the restoration of calcium. Injury to the parathyroids might likewise be a causative agent in the slow recovery sometimes observed after larger doses of oxalate, since the increased deficiency of hormone, by the greater damage to the gland, would necessarily lessen the speed of replacement and thus delay the return of blood calcium to its normal concentration. The same mechanism would likewise account for the progressive decline in the amount of blood calcium after the injection of large doses of oxalate, namely, failure to replace the calcium bound by oxalate. The results, presented above, there-

fore, justify the conclusion that the function of the parathyroids form an important element and may, indeed, be the principal factor in the determination of the effects of oxalate on the changes in blood calcium.

SUMMARY

1. The subcutaneous injections of oxalate produced the following changes in serum calcium: A single dose of 20 mg. per kilo was without any effect, but a progressive decrease of calcium occurred when this amount was given daily over a period of several days. A dose of 40 mg. oxalate per kilo caused a very moderate decrease of calcium within half an hour after the injection, and was much greater, about four or five times, after an interval of one hour. Signs of recovery were observed about four hours after the injection, and the normal level of calcium was regained in about twenty-four hours. When the dose was increased to 80 mg. per kilo, depletion of calcium half an hour after the injection was considerably greater than after 40 mg. doses. A moderate rise was observed four to five hours later, and although improvement was marked, in most cases, on the following day, no change or even a further decline of calcium was observed in some cases. Complete recovery occurred about two days after the injection.

2. The effects of oxalate after intravenous injections. An average decrease of 20 per cent occurred about ten minutes after 20 and 30 mg. per kilo, and a reduction of approximately 33 per cent ten to seventeen minutes after 40 mg. per kilo. A moderate amount of recovery was observed about four to five hours after the injection of 30 mg. oxalate per kilo, which was much less when 40 mg. per kilo were given. Although the rise in serum calcium was considerable after 30 and 40 mg. per kilo on the day after the injection, it was much less after the latter amount. The reduction of the absolute amount of calcium after 70 to 120 mg. oxalate per kilogram was much greater, but the relative decrease was less than after smaller doses.

3. After extirpation of the thyroid and parathyroids, the average decrease of calcium caused by the intravenous injection of oxalate, of 30 and 40 mg. per kilo, was about a fourth of that produced by the same amounts in normal animals, but the amount of reduction by large doses, the maximum of which exceeded that given to normal cats, was less than half of that produced in normal cats.

4. The results were discussed and the explanation offered that the effects on blood calcium after small, repeated doses and after large single doses, of oxalate injected subcutaneously, were due to the injury which it caused to the parathyroid, and that variation in the amount of diffusible calcium, due to different amounts of parathyroid hormone, was the main, if not the only, factor in determining the wide range of reduction of calcium in normal animals after the intravenous injection of oxalate. The small decrease of blood calcium after the administration of sodium oxalate to thyroparathyroidectomized cats was attributed to the low content of diffusible calcium.

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FIVE HUNDRED AND NINETEEN VOGE BROMINE TESTS OF URINE FOR PREGNANCY*

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EXAMINATION of 519 specimens has shown that the Voge bromine test is far from a satisfactory test for the diagnosis or exclusion of pregnancy. Positive results were obtained in but 63 per cent of the pregnancy urines and negative results in only 84 per cent of the nonpregnancy urines.

Voge[1] in 1929 proposed a bromine test for pregnancy based on the principle which he states as follows: "In view of the fact that a connection has been established between the elaborated product of the pituitary and compounds of B-iminazolyl (histamine, histidin and tethelin), it was thought advisable to test for the presence of this substance in the urine of pregnant women." He applied Knoop's[2] bromine test for histamine to the urine in the following way: To 2½ c.c. of urine in a test tube add 1 c.c. of bromine water. Heat to boiling.

In a positive test the color changes to pink in an alkaline urine or brownish pink in an acid urine, the color fading rapidly. In a negative test the color remains unaltered. The bromine water is prepared by diluting one part of 2 per cent bromine water with two parts of tap water.

Voge reported the results of his test with 60 specimens, the diagnoses being established by Aschheim-Zondek tests on the same specimens of urine. As will be seen in Table I he obtained excellent results in this small series of cases. Dodds[3] in 1930 and Siddal et al.[4] in 1931 reported their results with the test. Their percentage of agreement was much lower than that of Voge as will be seen in Table I in which are tabulated the results of the three reports, as well as our results in 519 specimens.

TABLE I

AUTHOR	TOTAL SPECIMENS	PREGNANCY	POSITIVE		FALSE NEGATIVE		NOT PREGNANT	NEGATIVE		FALSE POSITIVE	
			NUMBER	PER CENT	NUMBER	PER CENT		NUMBER	PER CENT	NUMBER	PER CENT
Voge	60	25	24	96	1	4	35	33	94	2	6
Dodds	305	229	171	74.3	58	25.7	76	66	86.9	10	13.1
Siddal et al.	260	36	27	75	9	25	224	207	92	17	8
Young	519*	131	82	63	49	37	388	325	84	63	16
Total:	1144	421	304	77	117	23	723	630	89	92	12.7

*All but a few were two tube tests.

Dodds draws the following conclusions: "It is apparent that the test is not pathognomonic of pregnancy. The error of 25.7 per cent in the antenatal

*From the Laboratory Department, Mount Sinai Hospital.
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cases makes the test of little value. The error of 13.1 per cent in the nonpregnant group makes the test valueless in the diagnosis of pregnancy."

The 519 tests reported here were done somewhat differently than originally proposed by Voge. Two tubes, in place of one, each containing 2½ c.c. of clear or filtered urine were used and to one 0.25 c.c. of bromine water was added, and to the other 0.5 c.c. of bromine water.* The test with the smaller amount of bromine was performed, since it was noted that in some pregnancy urines of low specific gravity (1.015 and less) the test with 0.5 c.c. of bromine was negative whereas with less bromine a definite pink color developed.

An analysis of the results obtained with the test using 0.25 c.c. of bromine water showed that while it increased the percentage of positive results in pregnancy urine from 54 per cent to 63 per cent it also increased the percentage of false positive results in nonpregnancy urine from 12 per cent to 16 per cent.

The quantitative feature of the test is explained by Hunter[5] in a note on his modification of the Knoop test for histamine as follows: "The color produced is very soluble in an excess of bromine and in a dilute solution of histamine an initial excess may prevent all color development. The difficulty of adding just sufficient bromine water to slightly colored solutions has been overcome by adding a definite excess and washing out the excess with chloroform."

The simpler expedient of using two quantities of bromine also satisfies the quantitative requirements of the test.

A number of the positive results in our series were obtained in specimens from children. By deducting these tests and recalculating the percentage of false positive results, the total is 13 per cent which is similar to that obtained by Dodds whose specimens were all from women.

COMMENT

The Aschheim-Zondek and Friedman tests of urine for pregnancy are well known and their great accuracy and value have been well established by numerous workers. These biologic tests, however, requiring the use of mice or rabbits are expensive and time-consuming, and there is need for a simple chemical test for pregnancy. As reported above the Voge test is unsatisfactory for this purpose.

Von Mertz[6] in 1928 proposed a chemical test for pregnancy on blood serum using as a test reagent phosphotungstic acid ($H_3 PO_4 + 12WoO_3 + 12 H_2O$). He obtained 94.9 per cent agreement in 650 cases. Unfortunately this work has not been sufficiently confirmed and Kilduffe and Steinig[7] have recently (1932) reported unsatisfactory results with it. They obtained a very low percentage of positive results (10.4 per cent) in pregnancy and approximately an equal number of positive results with nonpregnancy cases.

It still remains a problem to detect by a simple chemical means some substance in the urine or blood characteristic of the pregnancy state.

*The bromine water was prepared as follows: A stock solution of bromine water was made by placing in a glass stoppered bottle 6 c.c. of bromine (under a hood) and 300 c.c. of distilled water. After shaking for about forty-five minutes, a few cubic centimeters of the bromine remained undissolved and the supernatant solution was decanted. The stock solution therefore was about 1 to 1½ per cent of bromine. The stock solution diluted further with two parts of tap water constituted the test bromine water. The test bromine water was found to lose strength quite rapidly and was not used for more than one week after its preparation.

CONCLUSION

Examination of 519 specimens has shown that the Voge bromine test is far from a satisfactory test for the diagnosis or exclusion of pregnancy. Positive results were obtained in but 63 per cent of the pregnancy urines and negative results in only 84 per cent of the nonpregnancy urines.

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PHYTOPHARMACOLOGY OF STOMACH WASHINGS IN VARIOUS DIGESTIVE DISORDERS AND PERNICIOUS ANEMIA*

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DURING the past ten years the senior author has been developing a department of pharmacology to which the name of *phytopharmacology* has been applied. In this new field a comparative study was made in regard to the effects of various drugs, poisons, and toxins on living animal preparations, on the one hand, and living plant tissues, or plant protoplasm, on the other. These studies have led to some very interesting conclusions. It was discovered that living plant protoplasm may be much more sensitive to certain poisons than living animal protoplasm; and this was found to be particularly true of poisons or toxins elaborated by animals. By the use of quantitative phytopharmacologic methods, the first experimental evidence of the presence of various toxic substances in body tissues in certain pathologic conditions was obtained. Macht and Lubin showed the presence of a menotoxin or toxic substance in the blood, saliva, milk, perspiration and other secretions of women at the time of catamenia;[1] and these findings have since been confirmed by other investigators.[2, 3, 4] Again, for the first time, Macht and Pels have demonstrated experimentally the presence of a toxic body in the blood serum and bullae of the baffling skin disease, pemphigus.[5] Perhaps the most interesting findings so far obtained by Macht and his collaborators have been those resulting from their studies on pernicious anemia, the first of which was published in 1926.[6] While making a routine examination of specimens of normal and pathologic blood sera from numerous patients, the senior author found that serum from pernicious anemia cases exhibited very marked and characteristic toxicity for living seedlings of *Lupinus albus* grown in

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plant physiologic solution. The toxin was not menotoxin because it was present in male patients suffering from pernicious anemia, but it was not present in other blood diseases such as secondary anemia, carcinoma, leucemia, etc. The phytopharmacologic method therefore not only furnished a contribution to the etiology of Addison's or primary anemia but also offered a method of establishing a differential diagnosis between pernicious anemia and other anemias simulating it.[7] Later investigations elaborating the phytopharmacologic method of study furthermore offered a convenient means of evaluating various methods of therapeutic procedure employed in the treatment of pernicious anemia.[8] The findings obtained by means of this method of study in various diseases have been confirmed by numerous investigators, notably by Tscherkes,[9] Tscherkes and Goldstein,[10] Tscherkes and Mangubi,[11] Etinger and Artinov,[12] Stern,[13] Herz and Weichbrodt,[14] and Macht and Pels.[15]

More recently, Macht has demonstrated the presence of the pernicious anemia toxin not only in the blood but also in the spinal fluid of such patients.[16] A comparative phytopharmacologic study of spinal fluids from various conditions had led to the discovery of a definite toxicity in the spinal fluid of patients suffering from true pernicious anemia. This method is already being employed by some physicians for differential diagnosis of neurologic signs and symptoms produced by pernicious anemia, on the one hand, and other conditions simulating it, on the other. These findings suggested the undertaking of the present investigation. It was deemed worth while to inquire whether the stomach washings obtained from various normal and pathologic conditions showed any difference in toxicity when examined by the new phytopharmacologic method.

METHOD

After various preliminary experiments the following method was adopted. The empty stomach of the patient was washed out with 100 c.c. of distilled water. This was done by introducing water through a clean stomach tube, which had been previously sterilized and washed free from any trace of antiseptic. The washings thus obtained were tested for their hydrogen ion concentration and then submitted for special examination to the pharmacologic laboratory. The phytopharmacologic method employed was the same as that used in the study of spinal fluid and blood.[17] While a one per cent solution of the blood serum was employed in these experiments, a 2 per cent solution of the stomach washings was made because of the more dilute condition of the specimens at the outset. Such a 2 per cent solution was made in a plant physiologic saline consisting of equal parts of Shive solution and distilled water.[18] Healthy young seedlings of *Lupinus albus* were placed in hard glass tubes containing such a liquid and the growth of their straight and well-defined single roots in normal physiologic saline was compared with the growth of roots of similar seedlings from the same crop and of approximately the same initial length, immersed in the physiologic solution of the stomach washing. The length of the roots was measured at the beginning of the experiment and twenty-four hours after the preparations had been placed in

a dark incubator and kept at a temperature of 21° C. The increment in length of the seedlings obtained with stomach washings was then compared with the increase in length of the normal controls and expressed as the *phytotoxic index.*

Complete details in regard to the technic of the phytopharmacologic test have been given in previous publications and need no repetition in this place. It may be well, however, to state emphatically that, for careful performance of the phytopharmacologic test, a great deal of training is required not only in ordinary zoöphysiologic and zoöpharmacologic methods but also in the laboratory methods pertaining to plant physiology and botany. A beginner expecting to succeed in such work on the first attempt will more than likely be disappointed. Indeed, he may not even be able to germinate the seeds, much less to secure uniform seedlings suitable for testing. The seeds employed in the present work were those of the large variety of *Lupinus albus.* These seeds must not be too old or worm-eaten and should be soaked for twenty-four hours in tap water to insure their complete swelling before being planted in the proper germinating medium. Finely ground sphagnum moss is the most suitable medium for germinating seeds. It is of vital importance, however, that such moss should be free from acid matter and contain just the proper amount of moisture, namely, 75 to 80 per cent its weight. The sphagnum moss should be thoroughly aerated and not closely packed; and, after planting, the seeds should be set aside in the dark and kept at a temperature no higher than 22° C. When a higher temperature is employed, growth may be so greatly stimulated as to mask small differences produced by inhibitory substances in the various solutions. For the performance of the test, the seedlings should have roots measuring from 30 to 40 mm. in length. Rows of eight, or ten seedlings should be employed for control solutions and for each of the solutions to be tested. To insure greater accuracy it is well to select seedlings of as nearly the same length as possible for corresponding tubes in each row.

RESULTS

The total number of cases studied was 125. The average phytotoxic index obtained from stomach washings of 100 patients, either normal or exhibiting various gastrointestinal disorders without pernicious anemia, was found to be 86 per cent. The lowest reading in these cases was 75 per cent. Table I gives a detailed analysis of the first 59 of these cases. Here are set forth the age, sex, and color of the patient, social status, the amount of free acid, the total acidity and Wassermann findings, the clinical diagnosis or impression of each case, the x-ray findings and, finally, the phytotoxic indices, obtained independently in the pharmacologic laboratory and with no previous knowledge of the history and physical findings of the various cases. It will be noted that of the 59 cases 11 gave phytotoxic indices ranging from 76 to 80 per cent; 31 gave phytotoxic indices between 80 and 90 per cent; and 15 cases gave phytotoxic indices between 90 and 100 per cent. Two cases gave a reading over 100 per cent, the highest being 101 per cent. The phytotoxic indices in these cases and of all the stomach washings which were

TABLE I

NAME AND NUMBER	AGE	SEX	COLOR	SOCIAL STATUS	FREE ACIDITY	TOTAL ACIDITY	WASSERMANN	DIAGNOSIS	X-RAY	PHYTOTOXIC INDEX
M.W., H47411	54	F	W	M	4	28	Negative	Postoperative adhesions	Fluoroscope: colon spastic	85%
H.F., H57585	52	M	W	M	74	88	?	Impression: ulcer of duodenum (Paulson)	Spastic colon; no defects seen (B)	86%
E.H., H93408	35	M	W	S	18	28	Negative	Impression: cecal stasis	Nontubercular infiltration of both lungs	79%
H.D., J1204	35	M	W	S	16	34	Negative	Impression: possible ulcers; neurosis	Gallbladder series; no trace of dye in gallbladder, indicating some obstruction; no evidence of stones. Active hypermotile stomach; no defects in stomach or duodenum	90%
N.J., J24946	21	M	C	S	8	28	Positive 4+	Impression: reflex pylorospasm	Fluoroscope: some spasm at pyloric end and ptosis of transverse colon	89%
C.S., J40107	38	F	W	M	10	28	Negative	Psychoneurosis; hypertension; chronic constipation; functional dyspepsia; hypochlorhydria; aerophagia	Fluoroscope: moderate amount of cecal stasis; transverse colon prolapse slightly spastic	83%
T.H., J43492	27	M	C	M	6	24	Positive 4+	Functional dyspepsia	Fluoroscope; transverse colon, normal position; slightly spastic	76%
I.J., G10566	61	F	W	M	20	44	Negative		Fluoroscope: generalized visceroptosis; adhesions along colon; cyst form along clavicles; no dye enters gallbladder in series	90%
S.F., G98399	33	F	W	M	0	4	Negative	Impression: pelvic inflammatory disease; spastic colon	Fluoroscope: ptosis of colon; very atonic stomach; contour normal, cap normal	79%
R.G., G79393	41	F	W	M	10	26	Negative	Impression: cholecystitis	Gallbladder filled with dye and normal in outline; no stones seen (B)	99%
J.C., J48204	52	M	W		96 Histamine	100	?	(Some of history lost)	Fluoroscope: high spastic colon; second degree adhesions; right lower quadrant	76%
R.S., J59466	48	M	W	M	44	62	?	Postoperative adhesions; neurosis; postoperative hernia	Stomach normal in size, position and function; no lesion in stomach or duodenum	89%

TABLE I—CONT'D

M.L.,	J26071	43	F	W	W	0	4	Negative	Achylia gastrica; chronic tonsillitis; chronic constipation; ventral hernia	Colon completely filled; no defects seen; lower half of colon markedly spastic	83%
W.B.,	J49850	27	M	W	M	16	24	?	Irritable colon	Trace of dye in gallbladder; nothing abnormal seen	76%
M.S.,	G14543	67	F	C	M	30	40	Negative	Hypertension; arteriosclerosis; functional dyspepsia; chronic constipation; hypertrophic arthritis	Partial obstruction in lower end of esophagus; impossible to make diagnosis of neoplasm	82%
E.Y.,	J50343	26	F	W	M	38	56	Negative	Impression: psychoneurosis; constitutional inferiority; visceroptosis	Barium enema; normal large intestine; colon completely filled; no defects seen	90%
W.M.,	H81618	55	M	W	M	28	42	Negative	Chronic cholecystitis	Gallbladder does not fill with dye; very little dye in intestinal tract	95%
M.L.,	G95080	40	F	W	M	30	40	Negative		Normal concentration of dye in shape, size and position normal; no stones seen; contracts and empties normally	77%
A.F.,	J48877	34	F	C	M	12	20		Chronic gastritis	Fluoroscope: stomach normal position, normal tone; no defect in either curvature; no intestinal barium	89%
G.G.,	J49849		(G. I. history lost)					Positive 4+			77%
S.L.,	J44410	30	M	W	S	46	60	Negative	Cholecystitis	Fluoroscope: colon, good position; stomach, steer-horn type	88%
A.F.,	J46052	48	M	W	M	36	40	Negative	Functional dyspepsia	Fluoroscope: stomach very high; pulled up to right irritable pylorus	77%
L.W.,	J25490	31	F	C	M	15	30	Negative	Splanchnoptosis with atony; upper right quadrant adhesions	Fluoroscope: adhesions right upper quadrant; ptosis; mild lower quadrant adhesions	82%
H.B.,	J46328	46	M	W	M	8	28	Negative	Chronic appendicitis	Fluoroscope: stomach moderately prolapsed to right and downward; moderate spasticity of pyloric region; hepatic flexure prolapsed; cecal stasis; transverse colon spastic and prolapsed	86%

TABLE I—CONT'D

NAME AND NUMBER	AGE	SEX	COLOR	SOCIAL STATUS	FREE ACIDITY	TOTAL ACIDITY	WASSERMANN	DIAGNOSIS	X-RAY	PHYTOTOXIC INDEX
C.T., J46326	30	M	W	M	28	52	?	Irritable colon and reflex pylorospasm	Fluoroscope: stomach in good position; fair tone; no filling defect in pylorus or duodenum; transverse colon in good position	95%
I.G., J45630	43	F	W	W	20	30	?	Postoperative adhesions	Fluoroscope: transverse colon spastic in true pelvis; evidence of cecal stasis; stomach, fish-hook type	80%
L.C., J37692	57	F	W	M	10	32	Negative	Hiccoughs; chronic constipation	Gallbladder filled with dye; no stones seen	88%
W.F., J44694	26	M	W	M	10	13	Negative	Visceroptosis		101%
C.S., J44018	35	M	W	M	60	66	?	Recurrent ulcer	No dye in gallbladder; pyloric end of stomach constricted and irregular; duodenal cap not filled, suggesting ulcer	91%
A.S., J43847	20	M	W	S	54	78	Negative	Chronic appendicitis	Fluoroscope: bell-like, atonic stomach; cap, normal; large intestines, normal	95%
C.F., J43602	18	M	C	S	10	50	Negative	Functional dyspepsia; carious teeth; peptic ulcer (duodenal)	Very active stomach, occupying high position; no filling defect in stomach; duodenal cap slightly irregular; defect in duodenum; ulcer cannot be ruled out	78%
M.K., J43599	20	F	C	M	42	54	?	Impression: functional G. I. disorder with spastic G. I. tract	Large intestine, normal	82%
J.K., J43128	52	M	W	M	0	4		Gastric carcinoma; achylia gastrica	Annular filling defect in pyloric end of stomach, suggesting neoplasm; pyloric end quite irregular; large filling defect on greater curvature, which may be due to pressure from mass	88%
E.D.	30	F	W	M	40	52	Negative	Obesity	Gallbladder filled with dye and normal in appearance	89%

TABLE I—CONT'D

M.W.		49	F	W	M	6	16	?	Cardiac hypertrophy; myocardial insufficiency; chronic constipation; menopause; postoperative adhesions	Fluoroscope: first degree splanchnoptosis and atony	87%
J.R.,	J6777	38	M	W	M	26	40	?	Impression: spastic constipation; dermatitis	Intestinal tract filled with dye; none in gallbladder	80%
W.K.,	J45128	40	M	W	M	28	56	Negative	No G. I. condition		94%
C.C.,	U19982	38	F	C	M	18	40	Negative	Ptosis and atony; hyperchlorhydria	Fluoroscope: moderate degree of ptosis	78%
E.H.,	J45666	50	F	W	S	0	10	Negative	Impression: organic obstruction of pylorus, possibly carcinoma; myocarditis; cardiac arrythmia	Prolapsed hypertonic fish-hook stomach; no filling defect in stomach or duodenum	90%
D.S.,	J45499	23	F	C	M	18	42	Negative	Chronic appendicitis	Fluoroscope: lower right quadrant adhesions	88%
L.S.,	J46226	59	M	W	M	0	10	Negative	Achylia gastrica; gastrogenic diarrhea	Impossible to fill duodenal cap; no evidence of ulcer in stomach or duodenum; barium enema; normal large intestine	82%
M.B.,	H61540	42	M	W	S	64	82		Deferred?	Filling defect in duodenal cap due to ulcer	88%
I.L.,	H20529	41	F	W	M	38	60	?	Impression: psychoneurosis; functional dyspepsia	Fluoroscope: splanchnoptosis with atony	80%
L.S.,	H38052	43	M	W	M	12	22	?	Right upper quadrant adhesions; gastric neurosis	Very poor concentration of dye in galbladder; normal in size; contracts and empties normally	88%
F.N.,	J58438	28	F	W	W	14	36	Negative	Spastic colon; spastic constipation; rectal fissures; psychoneurosis	Gallbladder filled with dye and normal in outline; no stones seen; good expulsion. Fluoroscope: fish-hook stomach; complete prolapse of transverse colon	90%
B.S.,	J4952	21	F	W	S	10	28	Negative	Functional dyspepsia; dysmenorrhea (antiflexion subacute)	Fluoroscope: ptosed spastic colon	85%

TABLE I—CONT'D

NAME AND NUMBER	AGE	SEX	COLOR	SOCIAL STATUS	FREE ACIDITY	TOTAL ACIDITY	WASSERMANN	DIAGNOSIS	X-RAY	PHYTOTOXIC INDEX
M.K., U17993	35	F	W	S	0	4	Negative	Impression: no organic disease of G. I. tract	Fluoroscope: tremendously ptosed transverse colon; marked tenderness over entire abdomen	82%
M.G., U32693	39	M	C	M	0	10	Negative	Gastric ulcer	Defect in pyloric duodenal region, while not definite, sometimes suggests ulcer	85%
E.G., U13487	28	F	C	M			Negative	Exophthalmic goiter	No G. I. history to be found	88%
J.S., U33930	20	M	C	S	20	35	?	Impression: gastric ulcer	Faint concentration of dye in gallbladder; nothing abnormal seen	80%
J.P., G69165	68	W	W		22	32	?	Impression: constipation; old gastritis (alcoholic)	Fluoroscope: pylorus moderately hyperactive; duodenum visualized and showed no defect	96%
S.P., G65869	27	F	C	S	4	16	Negative	Obesity; oral sepsis; functional dyspepsia	Large hypertonic stomach lying in transverse position; very active and irritable; no filling defect in stomach or duodenum	100%
A.H., J35949	64	F	W	W	0	10	Negative	Achlorhydria; gallbladder disease	No evidence of dye in gallbladder; no stones seen. Failure of organ to concentrate dye indicates gallbladder disease; definite hypertrophic arthritis of lumbar vertebrae	82%
W.H., H38314	33	M	C	M	0	6	Negative	Functional dyspepsia	Fluoroscope: barium seen in transverse colon and splenic flexure; the latter is high and somewhat redundant. Stomach in good position; no irregularities in contour	76%
				Not with history						
M.S., J44536	54	F	C	M	8	40	?	Functional dyspepsia; spastic digestive tract	Fluoroscope; spastic G. I. tract	100%
E.F., J44685	20	M	W	S	44	66	?	Irritable G. I. tract	Fluoroscope: splanchnoptosis with atony	78%
E.S., J27565	22	M	W	S	8	50	Negative	?	Negative	87%
W.C., J43068	27	M	C	S	6	28	Negative	Gastric ulcer; hypertension	Fluoroscope and x-ray work not done	94%
F.B., U26585	43	F	W	S	24	34	Positive	Impression: syphilitic infection	?	84%

studied bore no relation whatever to the hydrogen ion concentration of the solutions used because a 2 per cent solution of such gastric washings in plant physiologic saline, which normally gave a reading of P_H 4.8 to P_H 5.0, was too small to change the hydrogen ion concentration to a degree sufficient to affect the growth of seedlings. The hydrogen ion concentrations of the solutions were studied by both colorimetric and potentiometric methods (Table I).

The phytotoxic indices of the cases discussed above are of interest both in regard to positive and to negative findings. Thus, for instance, it was very interesting to find that the stomach washings from several cases of carcinoma ventriculi showed no difference in toxicity as compared with those of other normal or functional gastric conditions. The most important findings, however, were made in connection with the comparative study of a series of true or essential achylias, on the one hand, and the achylias of pernicious anemia, on the other. Table II exhibits the results obtained. A total of eight cases

TABLE II

ESSENTIAL ACHYLIAS		ACHYLIAS OF PERNICIOUS ANEMIA	
CASE	PHYTOTOXIC INDEX	CASE	PHYTOTOXIC INDEX
1 C–l	82%	1 B–t	60%
2 S–n	95%	2 A–s	52%
3 L–n	83%	3 F–s	47%
4 S–f	82%	4 C–s	63%
5 F–d	79%	5 C–e	55%
6 H–k	82%	6 A–n	67%
7 K–s	88%	7 B–r	63%
8 N–l	90%	8 S–r	63%
		9 S–y	62%
Average	85%	Average	59%

of essential or true achylia was studied. These cases were investigated clinically and all proved to be of a severe type which did not respond with secretion of hydrochloric acid even after injections of histamine. In all these cases, morphologic and microscopic blood examination excluded pernicious anemia. It will be seen that the average phytotoxic reading given by the eight cases was 85 per cent, which was the same as the figure obtained in the series of 100 cases mentioned above. On the other hand, nine cases of pernicious anemia with complete achylia, studied by the authors, yielded very definite and different results. These cases were diagnosed as pernicious anemia on the basis of complete clinical, physical, and laboratory tests as well as on the basis of phytopharmacologic examination of the blood specimens. The average phytotoxic index given by the stomach washings from these nine cases was 59 per cent, a figure far below that obtained in other gastrointestinal conditions (Table II).

DISCUSSION

The findings described above in connection with the study of different samples of stomach washings from various clinical conditions are obviously of manifold interest to the physician. In the first place, the toxic reaction of the specimens obtained from cases of primary anemia promises to be of value

in the differential diagnosis of the achylia characteristic of that disease from other forms of achylia. In the second place, the peculiar toxic reaction produced by stomach washings from pernicious anemia cases is a property to be borne in mind in theoretical discussions in regard to the etiology of that disease. In the third place, the present findings have an added interest in connection with the treatment of pernicious anemia since the relative toxicity of stomach washings from a given patient, before and after instituting any special method of therapy, will furnish an index as to its efficiency.

Problems of gastric acidity have been brought into renewed prominence by the current intensive studies of pernicious anemia and of cancer of the stomach. As is well known, both these diseases are characterized by anacidity or achylia. Moreover, Polland and Bloomfield[19] have recently called attention to the surprising number of persons presenting an inexplicable anacidity of the stomach. Consideration of the different types of achylia or anacidity should make any new method of differentiating between the various forms especially welcome. Thus, the recent introduction of histamine as a potent stimulus to the secretion of free acid by the gastric mucosa has been of considerable aid in classifying various forms of anacidity. The phytotoxic test promises to be another such aid because phytopharmacologic examination of stomach washings indicates a distinct and marked difference between essential achylia, resistant to histamine injections, and that of pernicious anemia. What light these findings may shed on the etiology of primary anemia we cannot say at present but the experimental data are none the less valuable. In this connection it may be well also to bear in mind the more or less successful results obtained in the treatment of pernicious anemia with desiccated stomach mucosa. It is hoped that the present report will stimulate further investigation along phytopharmacologic lines.

SUMMARY

1. A phytopharmacologic examination, according to a standardized technic, was made of stomach washings obtained from 125 clinical cases, who had received no food for at least twelve hours.

2. The average phytotoxic index of one hundred patients not suffering from achylia was found to be 6 per cent.

3. A few cases of gastric carcinoma showed no difference in toxicity from other clinical conditions of a benign or functional character.

4. The average phytotoxic index for nine cases of definite pernicious anemia with no free hydrochloric acid was 59 per cent.

5. The average phytotoxic index of eight cases of essential achylia, which failed to respond with secretion to injections of histamine, was found to be 85 per cent.

6. The difference in phytotoxic effects between stomach washings obtained from cases of pernicious anemia, on the one hand, and essential achylia, on the other, promises to be of clinical value in differentiation of the two conditions and warrants further studies along the lines described above.

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METHYLENE BLUE AS AN ANTIDOTE FOR ANILIN DYE POISONING*

Case Report With Confirmatory Experimental Study

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THE recent press and technical journal notices regarding the use of methylene blue in the treatment of cyanide and carbon monoxide poisoning influenced the writers to try this procedure in a case of anilin dye poisoning, the details of which appear in the following report.

A male university student, aged twenty-three, while engaged in experimental work in a chemical laboratory, handled with his bare hands large quantities of para bromanilin and parabromorthosulphanilic acid. Intimate contact with the dye began about ten o'clock in the morning. In one portion of the experiment, while evaporating the material over an open flame, he inhaled the fumes for about an hour. At the end of this time it was noticed that his skin was becoming discolored. About twelve-thirty he left the laboratory and drove to his home. On the way he experienced a feeling of dizziness which gradually changed to severe headache. About 3 P.M. stupor developed. When seen at 4 P.M. the patient was semicomatose, had a severe headache, and the skin showed a discoloration suggesting an intense cyanosis. The color approximated a mauve lavender.

At this time the nature of the poisoning agent was not definitely known, and because of the grave condition of the patient, it was decided to remove him to the hospital and to try experimentally the methylene blue treatment as used by Geiger.

The patient was admitted to the Highland Hospital about 4 P.M. He was given at once, intravenously, 600 c.c. of 18 per cent glucose solution, plus 100 c.c. of 1 per cent methylene blue. Soon after the injection of this mixture was begun, the skin pigmentation began to disappear. In one hour the lips and nails became a normal pink color. The headache rapidly subsided. About 11 P.M. the patient was greatly improved and without headache. The skin, so far as could be observed, was normal in color.

Before instituting any treatment, and at irregular intervals thereafter, specimens of blood were taken for spectroscopic and other examinations. These disclosed that there was no disturbance in hemoglobin, red cell, white cell, or differential count. Blood sugar and blood nitrogen were also normal. The urine was normal both chemically and microscopically. A complete physical examination was made, which, except for the discoloration of the skin, was without significance. The pyrotannic test of the first sample of blood for carbon monoxide was normal.

*From the Highland Hospital.

The spectroscopic studies of the blood are of interest and significance, and are here given in detail. All blood samples were obtained from the median basilic vein. The first one, taken before treatment, had a distinct chocolate color. One part of this sample was diluted with 49 parts of distilled water, resulting in a reddish brown solution. This was examined spectroscopically, and showed the characteristic absorption bands of neutral methemoglobin between the C and D lines. A sample of blood taken twenty-four hours later, diluted as above, and examined spectroscopically, did not show any evidence of methemoglobinemia, but gave the characteristic absorption bands of oxyhemoglobin. (Plate I.)

Since the patient had been handling two distinctly different anilin products, parabromanilin, and parabromorthosulphanilic acid, animal experiments were undertaken to determine which of the two, or whether both of the substances were responsible for the poisoning of the patient.

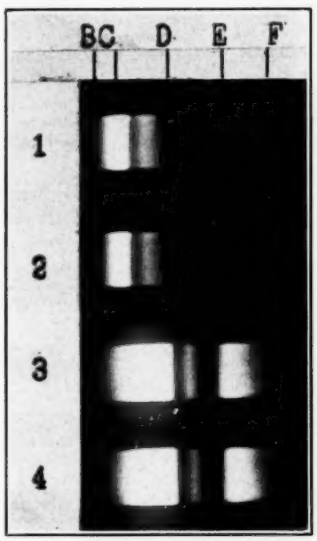

Plate I.—Showing spectrograms of blood of patient poisoned by contact with an anilin dye before and after the intravenous injection of methylene blue, as follows:

1. Blood of patient immediately before injection of methylene blue, showing pronounced neutral methemoglobin band.
2. Methemoglobin control.
3. Patient's blood one hour after injection of methylene blue showing normal oxyhemoglobin band.
4. Oxyhemoglobin of normal blood. Control.

Three rabbits were used. Rabbit No. 1 was a control, and received an injection of the 1 per cent methylene blue. Rabbit No. 2 was treated with an application of parabromanilin, in the form of a paste which was rubbed thoroughly into the skin. Rabbit No. 3 was treated in a similar manner with parabromorthosulphanilic acid. Rabbits No. 2 and 3 were later given injections of methylene blue.

Animal No. 2, treated with parabromanilin, became profoundly ill. Spectroscopic examination of the blood showed a marked neutral methemoglobinemia, which cleared up very rapidly after the injection of methylene blue.

Animal No. 3 showed no evidence of poisoning from the application of parabromorthosulphanilic acid, aside from a slight irritation at the site of

application. Spectroscopic examination of the blood showed the absorption bands of oxyhemoglobin, but no methemoglobin. (Plate II.)

The materials and apparatus used for this test, and details of the experiment are as follows:

1. Parabromanilin, 5 grams. Fifty per cent solution of ethyl alcohol and distilled water sufficient to make a thin paste.

2. Parabromorthosulphanilic acid, 5 grams. Fifty per cent solution of ethyl alcohol and distilled water sufficient to make a thin paste.

3. Methylene blue (Merck) medicinal, 1 per cent watery solution.

4. A Bausch and Lomb Universal Spectrophotometer, with camera attachment.

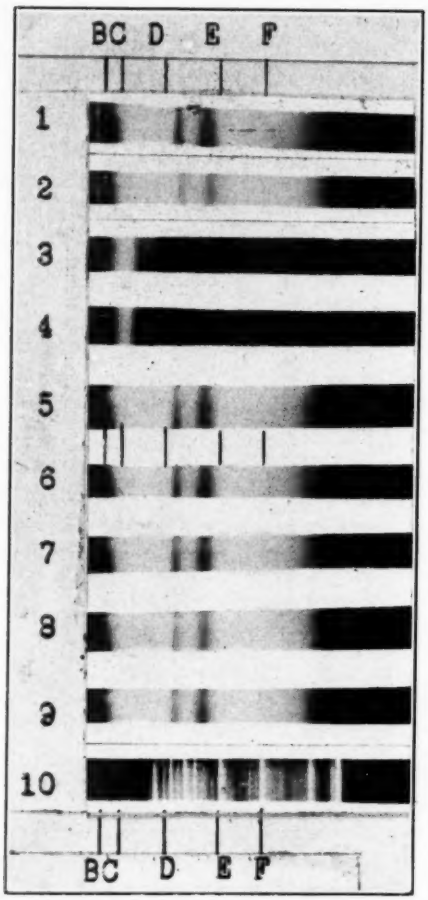

Plate II.—Showing spectrograms of bloods removed from experimental animals as follows:

1. Blood of Rabbit No. 1 before injection of methylene blue, showing the bands of oxyhemoglobin.
2. Blood of Rabbit No. 1 one hour after the injection of methylene blue, the oxyhemoglobin band being unaltered.
3. Blood of Rabbit No. 2 one hour after the application of parabromanilin to the skin, showing neutral methemoglobin.
4. Blood of Rabbit No. 2 at six hours, still showing a pronounced methemoglobin band.
5. Blood of Rabbit No. 2 one hour after injection of methylene blue. Methemoglobin band has been replaced by that of oxyhemoglobin.
6. Blood of Rabbit No. 2 after twenty-four hours, still showing oxyhemoglobin.
7. Blood of Rabbit No. 3 one hour after cutaneous application of parabromorthosulphanilic acid. Oxyhemoglobin present.
8 and 9. Same blood four and five hours later. Blood apparently not affected by the treatment.
10. Mercury arc.

Rabbit No. 1. Male, weight 1725 grams, given 1 cubic centimeter of the methylene blue solution. Samples of blood were taken before, and at one hour and four hours after injection. Spectroscopic examination showed oxyhemoglobin bands, but no methemoglobin. (See Plate II, Spectra 1 and 2.)

Rabbit No. 2. Male, weight 1880 grams, parabromanilin paste poured on animal's back and rubbed in well with wooden spatula. At the end of 30 minutes the animal was in distress, having an accelerated heartbeat, and quickened breathing. At the end of the first hour, the rabbit was very ill, and showed evident collapse. A sample of blood taken and diluted with 49 parts of distilled water gave a reddish brown solution, which on spectroscopic examination showed a definite absorption band of methemoglobin. (See Plate II, Spectrum 3.)

At five hours the rabbit was much worse, weaker, unable to sit up in normal position, and leaning against the side of the cage for support. Breathing rapid. Marked cyanosis of

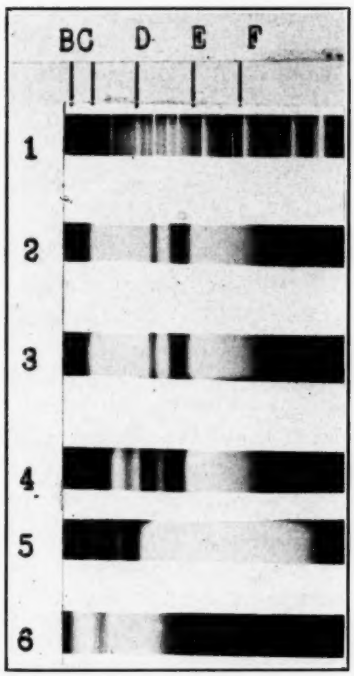

Plate III.—Showing spectrograms of control tests as follows:
1. Mercury arc.
2. Rabbit's blood before injection of methylene blue, normal oxyhemoglobin.
3. Rabbit's blood one hour after injection of methylene blue in twice the proportionate amount given to the patient, showing normal oxyhemoglobin.
4. Test tube mixture of normal blood and methylene blue. This seems to be a composite of the bands of oxyhemoglobin and methylene blue.
5. Dilute watery solution methylene blue.
6. Methemoglobin control.

ears. Second sample of blood taken, diluted as before, and examined spectroscopically. Blood chocolate color. Watery solution brown. Neutral methemoglobin bands very marked.

At six hours, rabbit very ill, and with no apparent improvement. Methemoglobin bands very strong. Rabbit given 1 cubic centimeter of methylene blue intravenously. (See Plate II, Spectrum 4.)

At seven hours, rabbit much improved, almost normal. Blood taken as before, and diluted with water. Blood normal in color, gives bright red solution. Spectroscopic examination shows normal oxyhemoglobin bands, no methemoglobin. (See Plate II, Spectrum 5.)

During this entire experiment the parabromanilin paste was not removed from the animal's back except as the rabbit itself licked and rubbed it way. After twenty-four

hours a fourth sample of blood was taken, and proved to be normal both in appearance and spectroscopic examination. (See Plate II, Spectrum 6.)

Rabbit No. 3. Male, weight 1460 grams, parabromorthosulphanilic acid rubbed into animal's skin, and blood taken at one, four, and five hours after application. Rabbit remained normal, and spectroscopic examination of blood showed no evidence of methemoglobinemia. (See Plate II, Spectra 7, 8, and 9.)

Since it has been reported by Wendel[1] that methylene blue injected intravenously produces methemoglobinemia, a male rabbit weighing 1650 grams was given 3.5 cubic centimeters of 1 per cent watery solution of methylene blue. Before the injection was completed, the bluish discoloration of the sclerae of the eyes began to appear. After fifteen minutes the sclerae were definitely blue, as was also the mucous membrane lining of the eyelids and mouth. At the end of one hour 0.5 cubic centimeters of blood was withdrawn, and diluted with 49.5 cubic centimeters of distilled water. This was examined spectroscopically. There was a marked shortening of the red end of the spectrum, but no evidence of the methemoglobin band. (See Plate III, Spectrum 2.) Blood was withdrawn again at the end of four hours, and examined with similar results. (See Plate III, Spectrum 3.)

For purposes of comparison, Plate III contains absorption spectra of oxyhemoglobin, neutral methemoglobin, and fresh blood mixed with methylene blue. (See Plate III, Spectra 1, 5, and 4.)

SUMMARY AND CONCLUSIONS

A case is described of anilin dye poisoning in a human being who had been handling parabromanilin and parabromorthosulphanilic acid, absorption presumably taking place from the skin. Intense signs and symptoms of pigmentation of the skin, headache, stupor, nausea, and vomiting rapidly supervened. These were promptly relieved by the intravenous injection of 1 per cent solution of methylene blue. Chemical, microscopic, colorimetric, and spectroscopic examinations were made of the bloods of both patient and experimental animals to determine the effect of cutaneous applications of the materials in question. It was definitely shown by spectroscopic studies of the blood that the poisoning in this case was due to parabromanilin, and that the parabromorthosulphanilic acid was nontoxic when applied to the skin.

The animal experiments indicated that methylene blue injected intravenously into a rabbit in approximately twice the strength of that given to the patient did not affect the absorption spectrum of normal blood, but if methylene blue be mixed with normal blood in vitro, the spectrum from such a mixture seems to be a composite of the spectra of methylene blue and normal blood. (See Plate III, Spectrum 4.)

A search of the literature fails to reveal a case of anilin dye poisoning resulting in methemoglobinemia in which the methemoglobin spectrum was changed to that of oxyhemoglobin with prompt clearing up of toxic signs and symptoms following the injection of methylene blue.

A brief review of the literature as to the use of methylene blue as an antidote is pertinent. The first to employ it thus was Sahlin of Lund, in 1926, who administered it in a case of cyanide poisoning, Eddy confirming

Sahlin's work in 1930 and 1931, as cited by Hanzlik.[2] Geiger was apparently the first to make clinical application of the procedure. For a statement as to the history and priority of the method, the reader is referred to Hanzlik's communication.

Brooks[3] found that methylene blue shortened the time of recovery from hydrocyanic acid poisoning to about one-third, and that from carbon monoxide to about one-half that of untreated animals. The results suggest that there is an activation of oxygen by methylene blue which manifests itself when aerobic oxidation is interfered with, and that it could be used advantageously in cases of hydrocyanic acid or carbon monoxide poisoning.

Geiger[4] reports three cases of cyanide poisoning ending in death, where the usual restorative methods failed. In a fourth and similar case, the patient was saved by the intravenous use of methylene blue, the treatment being suggested in this case by Doctors P. J. Hanzlik and C. D. Leake, of the University of California Medical School. Geiger later reports two cases of carbon monoxide poisoning treated with favorable results by the use of methylene blue.

A fourth report is by Nass,[5] who employed methylene blue in conjunction with carbon dioxide, oxygen inhalations, and other remedies in a case of illuminating gas poisoning, with successful outcome.

Since the preparation of this manuscript several communications have appeared in the literature, reporting the successful use of methylene blue in carbon monoxide poisoning. Haggard and Greenberg[6] sound a note of warning against the employment of methylene blue as an antidote. They assert that its use is fraught with danger, and support by chemical experiment Wendel's contention that methylene blue forms methemoglobin in the blood.

Our clinical observations on this point do not sustain Haggard and Greenberg's conclusion. This investigation throws no light on the mechanism of the methylene blue action in the blood. That in itself is an interesting problem which awaits solution.

NOTE: The writers wish to acknowledge with sincere thanks the assistance of the technical research division of the Bausch and Lomb Optical Company.

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A STUDY OF THE RED BLOOD CELL COUNT AND HEMOGLOBIN IN THE ADOLESCENT MALE*

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THE necessity for establishing the normal red blood cell count and hemoglobin standard of the peripheral blood for the various age groups has become evident with the growing appreciation of the influence of various factors on the cell concentration in the circulating blood. Many of the standards which had been accepted were made without the proper critical data as to their source or accuracy. Accurate observations by Haden,[1] Rud,[2] Gram and Norgaard,[3] Bing,[4] Williamson,[5] Komocki,[6] Osgood,[7] Wintrobe[8] and others have been published, based on blood determinations carried out with painstaking technic in large series of individuals. As a result of their investigations, normals have been established for the various adult age groups. To these we wish to add the results obtained in a series of 100 boys in the adolescent stage.

The subjects of our study were healthy male students between the ages of twelve and seventeen years. From the data collected from various other studies, we have no reason to assume that any organic disease was present. All subjects were American born and the majority resided in the Great Lakes region all their lives.

The examinations were done in the fall of the year and throughout the entire day so that the averages eliminate diurnal variations.

The specimens of blood were obtained from punctures of the lobe of the ear. The first drop was discarded, the succeeding drops flowed freely so that no squeezing was necessary. The red blood cell counts were made on a Neubauer-Levy counting chamber (new ruling, U. S. Bureau of Standards). The pipettes used were checked by the U. S. Bureau of Standards. The hemoglobin was calculated in a Sahli hemoglobinometer in the usual manner with N/10 hydrochloric acid for one minute, diluted with distilled water, and compared with a colored rod standard. In compiling the results to be discussed, the Sahli hemoglobinometer in which 14 gm. of hemoglobin were equivalent to 100 per cent, was used because of its convenience. The instruments were calibrated and checked in accordance with the Van Slyke gas method[9] for the estimation of hemoglobin.

RED BLOOD CELL COUNTS

As far as can be determined no large series of cases have been reported concerning the average red cell count in the adolescent stage. Our average in 100 boys between the ages of twelve and seventeen years was 4.71 million red

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blood cells per c.mm. Fig. 1 shows that 94 per cent of the cases ranged between 4 and 5.6 million red blood cells per c.mm., while 6 per cent of the cases were outside of this range. Thirty-one boys had counts of more than 5 million red blood cells per c.mm., and 4 had counts less than 4 million red blood cells per c.mm. The lowest count was 3.28 million red blood cells per c.mm., and the highest count was 5.87 million red blood cells per c.mm.

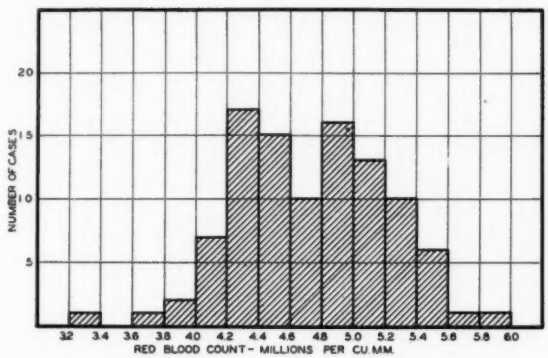

Fig. 1.—Distribution of red blood cell count in 100 boys between ages of twelve and seventeen years.

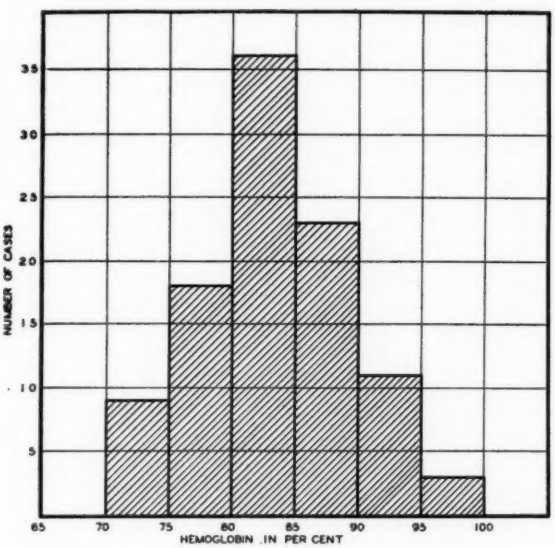

Fig. 2.—Percentage distribution of hemoglobin of 100 boys between the ages of twelve and seventeen years.

HEMOGLOBIN CONTENT

The inaccurate methods for the estimation of the hemoglobin content cannot be stressed too often. In a recent article[10] the fallacy of individual estimations was also pointed out. Any hemoglobinometer may be employed as long as it is calibrated in grams of hemoglobin per 100 c.c. of blood. From this scale the percentage content of hemoglobin can be easily computed. Inasmuch

TABLE I

OBSERVATIONS ON THE BLOOD IN ONE HUNDRED ADOLESCENT MALES

SUBJECT	AGE	RED BLOOD CELLS MILLIONS PER C.MM.	HEMOGLOBIN		C. I.	HEMOGLOBIN COEFFICIENT	
			%	GM.		%	GM.
1	12	4.44	82	11.48	0.92	91.0	12.74
2	12	4.38	75	10.5	0.85	82.5	11.55
3	12	4.82	71	9.94	0.74	73.8	10.36
4	12	4.28	85	11.90	1.00	98.6	13.80
5	12	5.41	75	10.50	0.70	69.0	9.66
6	12	4.50	81	11.34	0.90	89.1	12.46
7	12	4.18	81	11.34	0.97	97.2	13.58
8	12	5.38	80	11.20	0.75	73.6	10.29
9	13	4.53	75	10.50	0.83	82.5	11.62
10	13	5.23	80	11.20	0.77	76.8	10.28
11	13	4.55	70	9.80	0.78	77.0	10.78
12	13	4.01	81	11.34	1.00	99.2	13.86
13	13	4.82	82	11.48	0.85	86.0	12.04
14	13	4.62	85	11.90	0.92	91.8	12.88
15	13	5.06	83	11.62	0.82	82.0	11.48
16	13	5.55	83	11.62	0.75	75.5	10.57
17	13	5.04	76	10.64	0.75	74.4	10.36
18	13	4.40	83	11.62	0.94	94.3	13.16
19	13	4.76	83	11.62	0.87	87.9	12.32
20	13	4.86	85	11.90	0.87	85.0	11.90
21	13	4.84	84	11.76	0.87	87.3	12.18
22	13	4.36	83	11.62	0.95	96.2	13.44
23	13	5.87	70	9.80	0.60	60.2	8.40
24	13	4.80	85	11.90	0.88	88.4	12.39
25	13	5.20	80	11.20	0.77	76.8	10.78
26	13	5.20	85	11.90	0.81	81.6	11.41
27	13	4.37	74	10.36	0.85	85.0	11.48
28	13	4.63	83	11.62	0.90	89.6	12.53
29	13	4.80	80	11.20	0.83	83.2	11.62
30	13	4.04	81	11.34	1.00	101.0	14.14
31	13	4.80	76	10.64	0.79	79.0	11.06
32	13	3.84	70	9.80	0.92	91.7	12.91
33	13	3.99	83	11.62	1.03	103.7	14.59
34	13	4.92	81	11.34	0.82	81.0	11 34
35	13	5.05	82	11.62	0.81	81.0	11.34
36	13	3.73	81	11.34	1.08	108.0	15.12
37	13	4.28	71	9.94	0.83	82.3	11.55
38	13	4.45	78	10.92	0.87	87.0	12.18
39	13	5.30	78	10.92	0.73	74.8	10.50
40	13	4.33	82	11.48	0.95	95.1	13.30
41	13	5.28	89	12.46	0.85	83.6	11.69
42	14	4.24	97	13.58	1.14	114.0	16.31
43	14	5.06	85	11.90	0.84	85.0	11.90
44	14	4.94	87	12.18	0.88	87.8	12.32
45	14	5.21	87	12.18	0.83	83.5	11.69
46	14	4.31	81	11.34	0.94	93.9	13.16
47	14	4.24	75	10.50	0.89	90.0	12.60
48	14	5.10	88	12 32	0.86	86.2	12.04
49	14	4.22	82	11.48	0.97	98.4	13.72
50	14	5.22	78	10 92	0.75	74.8	10.50

TABLE I—CONT'D

SUBJECT	AGE	RED BLOOD CELLS MILLIONS PER C.MM.	HEMOGLOBIN		C. I.	HEMOGLOBIN COEFFICIENT	
			%	GM.		%	GM.
51	14	5.07	85	11.90	0.84	83.3	11.62
52	14	5.08	78	10.92	0.77	76.4	10.71
53	14	4.71	83	11.62	0.88	87.9	12.32
54	14	4.79	75	10.50	0.78	78.0	10.92
55	14	5.10	82	11.48	0.80	80.3	11.77
56	14	4.05	78	10.92	0.95	96.0	13.65
57	14	4.59	79	11.06	0.85	85.3	11.97
58	14	5.13	82	11.48	0.80	80.3	11.27
59	15	4.32	70	9.80	0.81	81.4	11.76
60	15	4.52	82	11.48	0.91	90.2	12.74
61	15	4.0	74	10.36	0.92	92.0	12.88
62	15	5.40	90	12.60	0.83	82.8	11.62
63	15	5.51	89	12.46	0.81	80.9	11.34
64	15	4.39	79	11.06	0.89	89.0	12.18
65	15	4.96	84	11.76	0.85	85.0	11.76
66	15	4.99	88	12.32	0.88	88.0	12.32
67	15	5.05	74	10.36	0.73	72.5	10.15
68	15	5.45	93	13.02	0.85	85.5	11.97
69	15	4.44	90	12.60	1.01	101.0	14.14
70	15	4.44	87	12.18	0.98	98.0	13.37
71	15	4.90	86	12.04	0.87	86.0	12.04
72	15	4.89	95	13.30	0.98	97.0	13.30
73	15	4.56	87	12.18	0.95	93.9	13.16
74	15	4.84	93	13.02	0.96	96.7	13.51
75	15	4.42	85	11.90	0.96	96.0	13.09
76	15	5.69	81	11.34	0.71	69.6	9.73
77	15	4.68	93	13.02	1.00	99.0	13.86
78	15	5.48	93	13.02	0.84	84.6	11.83
79	15	5.14	86	12.04	0.84	84.2	11.76
80	15	4.40	79	11.06	0.89	89.0	12.46
81	15	4.27	83	11.62	0.97	96.2	13.44
82	15	4.32	83	11.62	0.96	96.2	13.44
83	15	4.91	83	11.62	0.84	83.8	11.76
84	15	4.14	82	11.48	1.00	98.4	13.79
85	15	4.15	75	10.50	0.91	90.0	12.60
86	16	4.70	90	12.60	0.95	96.3	13.44
87	16	4.45	94	13.16	1.05	103.4	14.59
88	16	5.31	94	13.16	0.88	88.2	12.32
89	16	4.60	85	11.90	0.92	91.8	12.88
90	16	5.05	89	12.46	0.88	87.2	12.18
91	16	4.24	85	11.90	1.00	100.0	14.28
92	16	3.28	83	11.62	1.27	126.0	17.43
93	16	4.67	84	11.76	0.88	89.0	12.46
94	16	5.19	92	12.88	0.88	89.3	12.53
95	16	4.63	93	13.02	1.01	100.4	14.07
96	16	5.21	97	13.58	0.93	93.1	13.02
97	16	4.87	78	10.92	0.80	79.0	11.06
98	16	4.45	88	12.32	0.99	99.0	13.86
99	17	4.38	79	11.06	0.89	90.0	12.60
100	17	4.34	88	12 32	1.02	102.0	14.28
Average		4.718	82.57	11.55	0.87	88.26	12.35

as the hemoglobin varies with sex, age, time of day, altitude, climate, and race, the necessity for definite standards for the various age groups is indicated.

In the 100 cases herein reported, the average hemoglobin estimation was 11.55 gm. per 100 c.c. of blood or 82.5 per cent (Sahli). The lowest value was 9.80 gm. or 70 per cent (Sahli) and the highest value 13.58 grams or 97 per cent (Sahli). Of all the estimations, 88 per cent were between the range of 75 and 95 per cent (Sahli) or 10.50 to 13.30 gm.; 9 per cent of the cases were less, and 3 per cent of the cases were more than this (Fig. 2). The average hemoglobin content of each red cell was 26^{-12} gm., which is slightly less than the value for adult males as computed by Osgood.[7]

INDICES

The color index refers to the amount of hemoglobin in the average cell, expressing the amount in proportion to the normal. Table I contains the figures from which the various indices have been computed. The average uncorrected

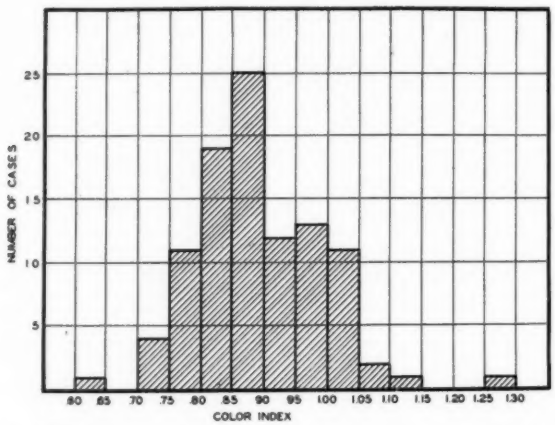

Fig. 3.—Color index distribution in 100 boys between the ages of twelve and seventeen years.

color index for the 100 cases is 0.87. The lowest index is 0.60 and the highest 1.27. In Fig. 3 it is noted that 83 per cent of the cases had a color index within the range of 0.80 to 1.15. Sixteen per cent of the cases had a lower color index and 1 per cent had a higher color index.

Curiously enough, if the hemoglobin coefficient be expressed as the percentage rather than the grams of hemoglobin per 5.0 million red blood cells, it has approximately the same figure as the color index. In computing the hemoglobin coefficient, it is noted that the average is 12.35 gm. per 100 c.c. which is a slightly higher value than the hemoglobin average. However, neither of these figures approaches the accepted standard for adults of 14 to 16 gm., which is recognized by the majority of investigators.

CONCLUSIONS

1. The average red blood cell count in males between the ages of twelve to seventeen years is 4.718 million per c.mm., the range being 3.28 to 5.87 million red blood cells per c.mm.

2. The average hemoglobin estimation is 11.55 gm. per 100 c.c. of blood or 82.57 per cent, the range being 9.80 gm. (70 per cent) and 13.58 gm. (97 per cent) per 100 c.c. of blood.

3. The average hemoglobin coefficient for the 100 cases is 12.35 gm. per 100 c.c. of blood.

4. The average color index for adolescent males is 0.87, the lowest index is 0.60 and the highest color index is 1.27.

5. If the hemoglobin coefficient be expressed in percentage rather than grams, it assumes approximately the same figure as the color index.

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THE BEHAVIOR OF LEAD IN THE ANIMAL ORGANISM*

III. Colloidal Lead Compounds

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INTRODUCTION

THE use of colloidal lead compounds in the treatment of malignant neoplasms has introduced a new factor into the problem of the physiologic behavior of lead. It may not be assumed that particulate lead compounds, injected intravenously, act in the same manner as those which are absorbed through the alimentary tract and the respiratory membranes. Thus, it is not surprising that some of the observations which have been made, following the administration of colloidal lead compounds, are not in harmony with previous experimental findings.

A review of the literature discloses certain facts upon which investigators are in agreement. Shortly after the injection of colloidal lead preparations the largest amounts of lead are found in the spleen and liver.[1, 2, 3, 4, 5, 6, 7] Frequently the lungs contain large amounts of lead. At this time the skeleton shows relatively low concentrations, while the kidneys, heart muscle, somatic muscle, brain, testicles and intestinal tract (without contents) show variable amounts. The blood stream loses its lead at a rapid rate after an injection. Bischoff[4] and his associates have described experiments in which virtually all the lead given as tri-lead orthophosphate was removed from the blood in two hours. Newman's[8] observations on horses showed that about 90 per cent of colloidal tri-lead orthophosphate disappeared from the blood in the first hour, while at the end of the second hour the blood was practically free of lead. In the case of colloidal lead, the removal from the blood stream was not so rapid, nor so complete, considerable amounts remaining in the blood sixty-six hours after the injection. Both chemical and histologic evidences point to the initial distribution of the colloidal compounds in the reticuloendothelial system,[6, 9, 10] while according to Henriques and Okkels[9] the administration of soluble salts promotes a contrasting deposition of lead in parenchymal cells of the liver and kidney.

The ultimate fate of the colloidal lead preparations in the tissues has not been studied adequately. Apparently, the importance of time as a factor in the dynamic processes of redistribution and excretion, has not been appreciated. Bischoff and Blatherwick[11] have mentioned fragmentary observations as evidence that injected lead orthophosphate induces an increased lead excretion. Todd[3] reports the study of the lead excretion of a patient treated with lead selenide. One would conclude from his results that the excretory

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rate for lead had been increased by the lead injections, but the effects were irregular. Millet[12] was unable to find evidence that the administration of colloidal lead orthophosphate altered the rate of lead excretion in the urine of patients. Newman, on the other hand, found a definite response in the horse,[8] and in patients,[13] after intravenous administration of colloidal lead orthophosphate, although the increase in the urinary lead concentration was small, and slow to make its appearance, as compared to the results obtained when colloidal lead had been injected.[8, 14]

We have studied the activity of certain insoluble particulate lead preparations in animals by methods similar to those previously employed in the study of soluble lead salts,[15] and tetraethyl lead.[16] We have made some observations on patients who have been treated for inoperable cancer with colloidal lead preparations. The animal experiments were designed to determine the character of the distribution of lead in the tissues, and the rate of its excretion not only immediately following injection, but also over subsequent periods during which the lead might be expected to migrate in the body.

EXPERIMENTAL METHODS

Five preparations were employed for intravenous administration.

1. A suspension of finely divided crystals of tertiary lead phosphate prepared by adding 1.5 per cent Na_3PO_4 to 0.5 per cent $PbCl_2$. The mixture was filtered at once, the precipitate was thoroughly washed on the filter paper but not dried, resuspended in distilled water, and used immediately.

2. A suspension of crystals of the secondary lead phosphate prepared in similar fashion, by using the secondary sodium phosphate as the precipitant.

3. Colloidal lead orthophosphate stabilized with gelatin prepared by the method described by Bischoff and Blatherwick.[11]

4. Colloidal lead orthophosphate procured from the Crookes Laboratories.

5. Colloidal lead from the same source.

The suspensions of crystalline phosphates were used to determine the influence of relatively large particles on the distribution of lead in animals, and to eliminate any uncertainty as to the chemical nature of the material injected. They were somewhat unsatisfactory as materials for intravenous injection, because of their tendency to block the syringe. Thus the amounts injected can only be estimated approximately. A small amount of blood was allowed to flow back into the syringe and after vigorous agitation of the contents of the syringe, the material was slowly injected. Death from embolism occurred in a few instances, but when the injection was carried out slowly the animals showed no ill-effects from any dosage employed. No attempt was made to determine the size of the particles of these suspensions.

Healthy full-grown rabbits were employed as experimental animals. Except for those that died as a result of the injection, they were sacrificed for analysis at suitable times, by bleeding from the heart under light ether anesthesia. Some of them were kept in metabolism cages from the time of injection until they were sacrificed, to follow their lead excretion. When this was done parallel observations were made on control animals. In other instances a control

period of excretion preceded the lead injection. Other animals kept for longer periods of time after injection, were introduced into the metabolism cages for a short time just before they were sacrificed. In this way information as to the magnitude of lead excretion at long intervals after treatment was provided.

The care of the animals, the excretory studies, the preparation of animal tissues, and the analytical procedures were carried out in the manner described in previous articles.[16, 17]

The observations on patients were made with the assistance of L. J. Schradin. The urine and feces were collected in containers provided by the laboratory. Precaution was taken against contamination of these samples from external sources. Tissues obtained at necropsy were washed in distilled water while fresh and stored in chemically clean jars.

RESULTS

We have given elsewhere the characteristic distribution of lead in the tissues of rabbits a long time after intravenous administration of lead salts.[15] Examples have appeared in earlier articles, illustrating the changes in the distribution[18] and excretion[16] of lead, which take place with the lapse of time following the intravenous injection of soluble lead salts into rabbits. The more complete data given in this paper will permit detailed comparison of the behavior of soluble lead salts with that of colloidal compounds. Table I shows the results of the analyses of the tissues of twenty-four rabbits that had received lead chloride intravenously. These animals died or were killed after various periods of time following the lead injections. Certain tissues were not analyzed separately. Rather, the attempt was made to select those organs which gave the greatest promise of information as to the distribution of lead. The mixed tissues which, for one reason or another, were not separated into individual samples, were grouped as one marked "remainder," or were discarded. The skin, ears, and feet were usually discarded because of the possibility of their external contamination, the ears as the sites of original injection being particularly under suspicion. In the case of several animals that were employed for excretory studies, there was no such question with reference to the ears because of the time interval between injection and death. Furthermore, the skin of these animals had been carefully cleansed at the beginning of the excretory observations, and again at the death of the animal. Such animals were analyzed in their entirety.

To clarify matters, the data are expressed in three forms. The upper section of the table shows the actual quantities of lead found by analysis; the middle section gives the calculated concentration in milligrams per one hundred grams of the tissue; the lower section relates the concentration in any one tissue to the concentration in the combined mass of tissue analyzed. This demonstrates the selective nature of the localization, and allows the analytical results in one animal to be compared with those in another regardless of the absolute amounts of lead present in the tissues.

Within a few minutes to two hours after intravenous injection of lead chloride, practically all the tissues examined showed the presence of lead. Un-

TABLE I

TABLE I

CHANGES IN THE AMOUNTS AND CONCENTRATIONS OF LEAD IN THE TISSUES OF RABBITS WITH THE LAPSE OF TIME FOLLOWING INTRAVENOUS ADMINISTRATION OF LEAD CHLORIDE SOLUTION

IDENTIFICATION NUMBER	243	258	236	252	238	237	257	242	256	251	264	244	250	253	255	206	171	172	254	203	205	176	249	278
Mg. of Lead Administered	6 mg. in 1 dose		15 mg. in 1 dose	12 mg. in 2 doses 1 wk. apart	34 mg. in 1 dose	28 mg. in 1 dose	12 mg. in 2 doses of 6 mg. each 1 wk. apart					4 mg. and 6 mg. 1 wk. apart	2 doses of 6 mg. 1 wk. apart	6 mg. and 4 mg. 1 wk. apart	2 doses of 6 mg. 1 wk. apart	15 mg. in 1 dose			2 doses of 6 mg. 1 wk. apart	15 mg. in 1 dose		26 mg. in 1 dose	12 mg. and 6 mg. 3 mo. apart	6 mg. in 1 dose
Time Interval After Last Injection	10 min.		30 min.		2 hr.	16 hr.	14 days			1 mo.		6 wk.				3 mo	5 mo.		5 mo.	7.5 mo.		9 mo.	11 mo.	
Lead in Milligrams Found in Tissues																								
Kidneys	1.41	0.19	trace	0.17	0.50	0.07	0.19	0.00	trace	0.00	0.08	0.09	0.00	trace	0.00	0.35	0.03	0.04	0.00	0.05	0.20	0.08	0.04	0.03
Liver	1.86	0.66	1.50	0.40	2.45	1.00	1.20	0.25	0.20	0.10	trace	0.24	0.09	0.00	0.10	0.37	0.19	0.08	trace	0.13	0.03	0.00	0.15	0.05
Spleen	0.00	0.00	0.60	trace	0.42	0.00	0.00	0.00	0.00	trace	0.00	0.00	0.00	0.00	0.00	0.09	0.00	trace	0.00	0.03	0.04	0.00	0.04	0.00
Fat	0.33	0.00	0.03	0.10	0.23	0.00	0.00	Lost	0.24	0.00	0.03	0.00	0.00	0.05	0.00	0.33	trace	0.06	0.07	0.18	trace	0.05	0.06	0.21
Central nervous system	0.03	0.00	0.05	0.00	0.74	trace	0.00	0.13	trace	0.00	0.00	0.07	0.00	0.00	0.00	0.09	0.00	0.11	0.00	0.12	0.03	trace	0.03	0.00
Blood	0.12	0.10	0.09	0.07	1.59	trace	0.12	0.17	0.08	0.00	0.06	0.00	0.07	0.03	0.03	0.03	trace	0.03	0.06	0.04	0.06	0.00	0.00	0.05
Muscle	0.61	0.50	0.75	0.15	2.50	0.28	0.36	0.35	0.16	0.27	0.14	0.11	0.00	0.12	0.23		0.35		0.04	0.21	0.05	0.05	0.04	0.11
Bone	0.95	1.05	1.25	2.70	1.76	1.40	0.98	3.35	2.60	0.77	2.45	3.13	1.98	2.20	2.00	4.24	1.11	1.05	1.08	1.71	0.37	0.71	0.69	0.07
Washed int. tract	0.42	0.54	0.84	0.18	1.77	0.57	0.10	0.14	0.00	0.05	0.06	0.00	0.00	trace	0.00	0.04	0.04	0.12	0.03	0.06	0.03	0.00	0.16	0.54
Skin								0.60					0.36		0.25	0.18		0.10		trace	trace	0.04		
Remainder								0.71				1.46	1.16		1.00	0.12		0.06	0.42				1.12	2.27
All tissues	5.73*	3.04*	5.11*	3.79*	11.96*	3.32*	2.95*	5.70	3.28*	1.19*	2.82*	5.10	3.66	2.40*	3.61	5.84	1.72*	1.65*	1.70	2.53*	0.81*	0.93*	2.33*	3.33*
Lead in Milligrams Per 100 Grams Tissue																								
Kidneys	10.33	1.19		0.81	2.17	0.37	1.00	0.00		0.00	0.44	0.60	0.00		0.00	2.92	0.18	0.29	0.00	0.38	1.82	0.80	0.17	0.17
Liver	2.14	0.47	1.69	0.37	1.99	1.06	1.29	0.25	0.27	0.18		0.25	0.12	0.00	0.11	0.44	0.21	0.09		0.11	0.06	0.00	0.12	0.05
Spleen	0.00	0.00	20.00	0.67	9.33	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	3.00	0.00		0.00	1.00	4.00	0.00	1.60	0.00
Fat	4.72	0.00	0.05	0.35	0.24	0.00	0.00		0.21	0.00	0.09	0.00	0.00	0.19	0.00	0.94		0.06	0.13	0.03		0.01	0.04	0.04
Central nervous system	0.21	0.00	0.33	0.00	4.35		0.00	0.93		0.00	0.00	0.44	0.00	0.00	0.00	0.69	0.00	0.79	0.00	1.00	0.21		0.14	0.00
Blood	1.33	2.50	1.00	0.70	8.37		1.41	0.19	0.11	0.00	0.05	0.00	0.08	0.04	0.03	0.07		0.04	0.08	0.04	0.09	0.00	0.00	0.03
Muscle	0.10	0.09	0.07	0.02	0.22	0.03	0.05	0.04	0.02	0.04+	0.02	0.01	0.00	0.02	0.02		0.05		0.01	0.02	0.01	0.01	0.01–	0.01
Bone	0.48	0.62	0.88	1.90	0.99	0.82	0.69	1.48	1.83	0.54	1.24	1.58	1.00	1.11	0.88	2.50	0.49	0.46	0.95	0.75	0.19	0.31	0.30	0.02
Washed int. tract	0.21	0.24	0.59	0.09	0.99	0.40	0.06	0.06	0.00	0.04–	0.03	0.00	0.00		0.00	0.02	0.02	0.09	0.02	0.04	0.03	0.00	0.07	0.27
Skin								0.21					0.14		0.06	0.08		0.04				0.02		
Remainder								0.17				0.18	0.24		0.18	0.02		0.01	0.10				0.08	0.13
Relative Concentration of Lead in Tissues Ratio $\frac{\text{Mg. per 100 Grams of Tissue}}{\text{Mg. per 100 Grams of combined Tissues}}$																								
Kidneys	20.75	4.43		2.95	3.22	1.64	4.02				2.23	2.88				7.33	1.51	2.86		4.00	34.30	18.70	2.60	2.30
Liver	4.28	1.76	5.12	1.36	2.95	4.73	5.18	0.96	0.97	1.68		1.22	0.67		0.85	1.24	1.80	0.93		1.10	1.13		1.84	0.65
Spleen			66.67	2.43	13.83											7.54				10.42	75.47		23.88	
Fat	9.47		0.15	1.25	0.36				0.74		0.44			0.86		2.37		0.56	1.22	0.34		0.28	0.66	0.57
Central nervous system	0.43		1.00		6.45			3.65				2.10				1.74		7.85		10.42	4.04		2.09	
Blood	2.68	9.33	3.00	2.55	12.40		5.67	0.72	0.40		0.26		0.45	0.19	0.21	0.18		0.43	0.74	0.46	1.70			0.44
Muscle	0.20	0.35	0.22	0.07	0.33	0.12	0.20	0.17	0.09	0.40	0.09	0.06		0.10	0.18		0.39		0.05	0.18	0.11	0.11	0.04	0.10
Bone	0.96	2.31	2.67	6.91	1.47	3.66	2.77	5.78	6.63	5.22	5.65	7.58	5.59	5.14	6.83	6.28	4.18	4.63	8.77	7.84	3.51	7.28	4.54	0.29
Washed int. tract	0.43	0.89	1.79	0.33	1.47	1.78	0.24	0.22		0.34	0.15					0.04	0.21	0.85	0.17	0.36	0.49		1.04	3.54
Skin								0.82					0.79		0.46	0.21		0.42				0.42		
Remainder								0.65				0.88	1.35		1.36	0.04		0.08	0.91				1.27	1.69

*Skin and/or mixed tissues ("Remainder") discarded.

doubtedly some of this lead was in the residual blood of these tissues, and since the blood was high in lead at this time, this factor is important. However, in some organs the concentration of lead was higher than would be expected from their blood content, while the reverse was true in others. In general the kidneys, liver, spleen, and the blood were especially high in their lead content, though there was a considerable variability in the relative positions of these solid organs. Muscle tissue was uniformly low in lead, and, with one exception, the bones. (The exception [Rabbit 252] is the result of the injection of 6 mg. of lead a week before the final injection.) The amounts in the bones are somewhat higher than would be expected immediately after injection. No doubt the vascular bone marrow was responsible for a certain quantity, but the additional factor of lead present before the experimental injection may not be ignored. We have found that the lead in normal, untreated rabbits varies in amounts from 0.29 mg. to 2.60 mg., as shown in Table II and Table III. The chief influence of such normal lead in these experiments is to magnify the lead content of the skeletal tissues.

The only significant difference in the distribution of lead sixteen hours after injection is seen in the result obtained on the blood of Rabbit 237, which showed only a trace of lead. The data of these experiments are not adequate to show the time at which the greater proportion of injected lead disappears

TABLE II

LEAD FOUND IN NORMAL RABBITS BY ANALYSIS OF ENTIRE CARCASS

IDENTIFICATION NUMBER	PB IN MG.	IDENTIFICATION NUMBER	PB IN MG.
133	0.00	93	1.40
111	0.29	C_2	1.50
119	0.45	89	1.70
P_1	1.10	98	1.70
C_1	1.23	121	2.60

TABLE III

THE DISTRIBUTION OF LEAD IN UNTREATED (CONTROL) RABBITS

IDENTIFICATION NUMBER	364	373	361
	Lead in Milligrams Found in Tissues		
Kidneys	0.00	0.00	0.00
Liver	0.00	0.00	0.00
Bile	0.00	0.00	0.00
Spleen	0.00	0.00	0.00
Fat	0.00	0.00	0.00
Central nervous system	0.00	0.00	0.00
Blood	0.00	0.00	0.00
Muscle	0.00	trace	0.00
Bone	0.00	0.04	0.68
Lungs	0.00	0.00	0.00
Heart	0.04	0.00	0.00
Intestinal tract with contents	0.05	0.07	0.06
Skin	0.10	0.08	0.00
Remainder	0.13	0.20	0.56
All Tissues	0.32	0.40	1.30

from the blood stream. From other experiments in which only a few tissues were analyzed, it is apparent that from sixteen to seventy-two hours are required to accomplish the result.

Gradual changes in distribution occur between the first and fourteenth days. After that time no obvious evidences of change are found in the analytical results. There is a gradual diminution in the gross amount of lead in the tissues, without any striking shift from one tissue to another. There is a considerable variability among the animals, but the trend is fairly uniform. The bones have absorbed their maximal absolute amounts by the end of two weeks. From this time on, the concentration of lead in the bones does not increase appreciably, either actually or in relation to the other tissues. The spleen apparently loses the greater portion of its lead during the initial two weeks, for large amounts are not found in the spleen of any animal after that time. The liver and the kidneys usually show relatively large amounts of lead. Fatty tissue, striated muscle and intestinal tract (without contents) contain small amounts with considerable regularity. Lead was found in the skin in every analysis, and in amounts which could not be attributed to external contamination. The analysis of the central nervous system did not yield lead regularly. The amounts were small in every instance except one.

Nearly 80 per cent of the animals killed fourteen days or more after the last lead treatment showed measurable amounts of lead in the blood. This fact demonstrates the mobility of lead in the bodies of these animals.

A few findings are not recorded separately. Samples of hair of Rabbits 171, 172 and 206, weighing 11 gm., 11 gm., and 15 gm. respectively, contained 0.00, 0.03 and 0.06 mg. of lead; the testicles, suprarenals, and heart of Rabbit 244, showed respectively 0.06 mg., 0.04 mg., and a trace; the uterus of Rabbit 278 showed a trace, while the suprarenals and heart showed 0.04 and 0.03 mg.; minute amounts of bile from Rabbits 249 and 278 failed to show lead.

The diminution in the actual quantities of lead found in the tissues as a result of excretory processes is best demonstrated by the amounts found in the entire carcasses of Rabbits 242, 244, 250, 255, 206, and 254 (see Table VII, showing the excretory data). It may be noted that after a period of five or six months, there is little evidence of a further drop in the total amount of lead. The explanation of this fact is plain, when reference is made to Tables II and III. An equilibrium had been established with an environment in which lead absorption was unavoidable. We shall return to this point in the data on excretion, but it must be stressed here, that the presence of such amounts of lead, months after its experimental administration, must not be interpreted as evidence of its retention by the tissues. It is the product of the two opposed processes of lead ingestion with food, and lead excretion.

Table IV presents the analytical data on two rabbits which received a suspension of lead orthophosphate intravenously, and on three rabbits similarly injected with secondary lead phosphate. The dosages indicated are approximations. The discrepancies between the recorded dosages and the

TABLE IV

CHANGES IN THE AMOUNTS AND CONCENTRATIONS OF LEAD IN THE TISSUES OF RABBITS WITH THE LAPSE OF TIME FOLLOWING INTRAVENOUS INJECTION OF TERTIARY AND SECONDARY LEAD PHOSPHATES FINELY DIVIDED IN WATER

IDENTIFICATION NUMBER	235	234	239	240	241
Mg. of Pb injected	100	200	100	100	100
Time interval after injection	1 day	3 days	1 day	3 days	37 days
Material injected	Aqueous suspension of $Pb_3(PO_4)_2$		Aqueous suspension of $PbH(PO_4)$		
	Lead in Milligrams Found in Tissues				
Kidneys	0.03	0.19	0.17	0.31	0.21
Liver	36.00	57.00	33.50	46.50	24.00
Spleen	1.23	7.63	1.70	3.75	5.88
Fat	0.00	0.31	0.05	0.11	0.02
Central nervous system	0.05	0.00	0.00	0.02	0.00
Blood	0.10	0.47	0.30	0.07	0.11
Muscle	0.19	0.11	0.11	0.25	0.14
Bone	1.95	7.00	0.59	3.75	14.50
Washed int. tract	0.03	0.26	0.05	0.14	0.32
All tissues*	39.58	72.97	36.47	54.90	45.18
	Lead in Milligrams per 100 Grams of Tissue				
Kidneys	0.18	1.36	0.85	1.72	1.75
Liver	46.15	57.00	40.85	44.28	28.57
Spleen	61.50	169.56	42.50	25.00	392.00
Fat		0.37	0.05	0.07	0.04
Central nervous system	0.31			0.12	
Blood	0.08	0.46	0.26	0.05	0.09
Muscle	0.02	0.01	0.01	0.03	0.02
Bone	1.15	4.12	0.33	2.21	10.21
Washed int. tract	0.02	0.10	0.03	0.06	0.14
All tissues*	2.38	4.58	2.42	2.95	3.25
	Relative Concentration of Lead in Tissues				
Ratio $\frac{\text{Mg. per 100 Grams Tissue}}{\text{Mg. per 100 Grams All Tissues}}$					
Kidneys	0.07	0.30	0.35	0.58	0.54
Liver	19.36	12.46	16.86	15.02	8.80
Spleen	25.80	37.06	17.54	8.48	120.73
Fat		0.08	0.02	0.02	0.01
Central nervous system	0.13			0.04	
Blood	0.03	0.10	0.11	0.02–	0.03
Muscle	0.01–	0.01–	0.01–	0.01–	0.01–
Bone	0.48	0.90	0.14	0.75	3.14
Washed int. tract	0.01	0.02	0.01	0.02	0.04

*The skin, mixed tissues, and other small tissues not enumerated have been discarded. Therefore the amounts of lead in the entire carcass of the animals are not available.

amounts of lead recovered are explainable on this ground, and for the additional reason that the lungs, skin, feet, ears, and the mixed tissues were not subjected to analysis.

The distribution of the finely divided insoluble lead salts is similar to that of the soluble salts during the first few hours, except that it shows less variation. In every case a large proportion of the lead was recovered from the liver. The amounts in the kidney were small, both in concentration and in the absolute, while the concentration in the spleen was uniformly high. The blood concentration was relatively low and did not obscure the character of

the distribution. (These animals were bled to the point of exsanguination, since none of them died in consequence of the administered lead.)

The time factor in the case of Rabbit 241, gives a special significance to the distribution of lead in its tissues. Thirty-seven days after the injection of secondary lead phosphate, over half of the lead found in the tissues was present in the liver, while less than a third of the recovered lead was found in the skeleton. The total lead recovered is equally remarkable. Little lead had been sufficiently mobile either to redistribute itself into other tissues or to be excreted. The behavior of this lead compound is therefore distinctly different from that of the soluble salts.

The results obtained after the injection of stabilized colloidal lead orthophosphate appear in Table V. Despite the higher dispersion of the particles of the lead compound, no difference in behavior was noted as compared with crystalline suspensions of lead phosphates. A more complete picture of the distribution of lead is presented in that a larger number of tissues appear in the analytical data, but the essential character of the slow migration of lead is unchanged. Again the liver is the chief depository for the injected lead, and again this organ retains a large proportion of its lead for thirty days. The bones slowly gain lead, though it is clear that at some time after the first week they begin to lose it more rapidly than they gain it. It appears that the lungs and the spleen give up all but a small amount of their initial lead, though this apparent trend may be due to the variability in the initial deposition of lead in these tissues. There is no question, however, that the liver and the spleen are more effective filters for the particulate lead than are the lungs and the kidneys.

The study of the activity of colloidal lead brings to light a series of changes in the distribution of lead which resemble those which occur when soluble salts of lead are injected intravenously. Table VI presents the facts. The initial distribution of lead is not remarkable. Rabbits 366 and 365 give a better concept of the early state of affairs than do Rabbits 369 and 368. (Rabbit 369 died from the injection with a high concentration of lead in its blood, while Rabbit 368 was found dead and could not be bled. The blood which was present in the tissues of these animals tends to obscure the picture.) Seven days after the injection, a significant loss of lead from the body had occurred, accompanied by a redistribution of lead in the tissues. Thus the kidneys show little lead, the amounts found in the liver are small in relation to the total lead content of the body, and the spleen shows only traces of lead. The bones, on the contrary, had gained more lead than they had lost. (The amounts of lead recovered from Rabbit 365, as well as the distribution of lead in the tissues, are atypical. This is not chance, but the consequence of lead present in the tissues before the experimental treatment, as shown by the fact that the recovered lead is greater than the amount injected.)

The analytical findings in Rabbit 363 give evidence of further changes in lead distribution. Rabbit 362 undoubtedly had lead in its tissues before treatment, but in addition to this factor its lead excretion was small as compared with Rabbit 363 (Table VII). The facts are evident, nevertheless, when the original amounts in the tissues are obtained by the summation of

TABLE V. CHANGES IN THE AMOUNTS AND CONCENTRATIONS OF LEAD IN THE TISSUES OF RABBITS WITH LAPSE OF TIME FOLLOWING INTRAVENOUS INJECTION OF COLLOIDAL LEAD PHOSPHATE PREPARATIONS IN GELATIN

IDENTIFICATION NUMBER	315	313	318	320	316	356	359	357	360	358
Mg. of Pb injected	±100	±100	±40	±100	±45	7.6	15.2	15.2	15.2	22.8
Time interval after injection	1 hr.	1 day	4 days	1 wk.	1 mo.	3 days	10 days	14 days	30 days	
Material injected	Colloidal $Pb_3(PO_4)_2$ prepared in Lab.					Commercial preparation of colloidal $Pb_3(PO_4)_2$				
Lead in Milligrams Found in Tissues										
Kidneys	0.75	0.08	0.09	0.08	0.11	0.00	0.00	trace	0.00	0.00
Liver	23.25	44.25	8.75	50.00	11.50	4.30	4.00	5.20	3.00	3.20
Bile	0.03	0.05	trace	0.00	0.00		0.00		0.00	0.00
Spleen	0.62	2.65	0.21	2.45	0.09	0.20	0.20	0.19	0.68	0.00
Fat		0.03	0.21	0.03	0.00					0.00
Central nervous system	0.19	0.05	0.05	0.03	0.04	0.00	0.00	0.00	0.00	0.00
Blood	33.00	0.07	0.08	0.05	0.04	0.00	0.00	trace	0.00	0.00
Muscle	3.30	0.42	0.24	0.19	0.14	0.05	0.00	0.10	0.00	0.00
Bone	6.20	24.00	16.50	32.00	12.00	0.58	3.80	2.30	1.60	1.90
Lungs	2.22	0.54	0.19	0.40	0.04	0.00	trace	trace	0.00	0.00
Heart	0.44	0.08	0.03	0.04	0.39		0.00	0.00	0.00	0.00
Washed int. tract	0.99	0.34	0.23	0.03	0.12	0.12	0.04	0.16	0.13	0.07
Contents of intestine	0.35	0.90	–*		–*					
Testicles	0.05									
Ovaries		0.07	trace	trace	0.00					
Suprarenals	0.04	0.06	0.04	trace	trace					
Uterus		0.10	0.03	0.00	0.04					
Skin	1.35	–*	0.26		–*	0.12	0.04	0.07	0.00	0.03
Remainder	9.60	6.00	6.00	7.50	1.00	0.28	1.17	1.44	1.28	0.35
All tissues	82.38	79.69	32.95	92.80	25.53	5.65	9.26	9.52	6.69	5.55
Lead in Milligrams per 100 Grams of Tissue										
Kidneys	6.25	0.73	0.55	0.62	0.69	0.00	0.00	0.00	0.00	0.00
Liver	27.10	57.50	9.11	43.80	16.20	6.63	4.93	8.14	3.33	3.05
Bile	6.00	3.33			0.00		0.00		0.00	0.00
Spleen	124.00	132.50	7.00	98.00	2.57	10.00	20.00	9.50	68.00	0.00
Fat		0.06	0.15	0.07	0.00					0.00
Central nervous system	1.46	0.44	0.33	0.15	0.21	0.00	0.00	0.00	0.00	0.00
Blood	43.48	0.12	0.07	0.03	0.03	0.00	0.00	0.00	0.00	0.00
Muscle	0.73	0.07	0.04	0.03	0.02	0.01	0.00	0.02	0.00	0.00
Bone	4.37	16.90	11.62	18.83	5.29	0.47	2.31	1.67	0.94	1.19
Lungs	24.67	5.40	1.41	2.86	0.27	0.00		0.00	0.00	0.00
Heart	8.80	1.46	0.43	0.57	4.88		0.00	0.00	0.00	0.00
Washed int. tract	0.58	0.30	0.14	0.02	0.05					
Contents of intestine	0.09	0.53								
Testicles	3.33									
Ovaries		14.00			0.00					
Suprarenals	13.33	12.00	5.00							
Uterus		1.33	0.27	0.00	0.31					
Skin	0.79		0.08			0.07	0.02	0.03	0.00	0.01
Remainder	1.78	0.82	0.52	1.20	0.11	0.04	0.18	0.19	0.13	0.03
Relative Concentration of Lead in Tissues—Ratio					*Mg. per 100 Grams Tissue*					
					Mg. per 100 Grams Total Carcass					
Kidneys	1.17	0.17	0.42	0.14	0.72	0.00	0.00	0.00	0.00	0.00
Liver	5.07	13.50	7.04	10.08	16.91	23.50	11.51	20.42	14.60	17.25
Bile	1.12	0.79			0.00		0.00		0.00	0.00
Spleen	23.20	31.20	5.40	22.50	2.68	35.50	46.95	23.90	298.00	0.00
Fat		0.01	0.11	0.01	0.00					0.00
Central nervous system	0.27	0.10	0.26	0.04	0.22	0.00	0.00	0.00	0.00	0.00
Blood	8.12	0.03	0.05	0.01	0.03	0.00	0.00	0.00	0.00	0.00
Muscle	0.14	0.02	0.03	0.01	0.02	0.04	0.00	0.05	0.00	0.00
Bone	0.82	3.98	8.98	4.33	5.52	1.67	5.52	4.19	4.13	6.73
Lungs	4.60	1.27	1.09	0.66	0.28	0.00	0.00	0.00	0.00	0.00
Heart	1.65	0.34	0.33	0.13	5.09		0.00	0.00	0.00	0.00
Washed int. tract	0.11	0.07	0.10		0.05					
Testicles	0.62									
Ovaries		3.30			0.00					
Suprarenals	2.50	2.82	3.86							
Uterus		0.31	0.21		0.32					
Remainder	0.33	0.19	0.40	0.28	0.11	0.14	0.41	0.47	0.55	0.18

*Discarded. Not included in analytical results.

the lead recovered from the tissues and the excreta. On this basis, Rabbit 362 had 13.7 mg. of lead in its tissues at the outset. This was redistributed in its tissues in substantially the manner shown by other animals of the series, but only 6.45 mg. were excreted. Rabbit 363 had 11.87 mg. originally but in the process of redistribution, it lost 8.88 mg. through excretion.

TABLE VI

CHANGES IN THE AMOUNTS AND CONCENTRATIONS OF LEAD IN THE TISSUES OF RABBITS WITH THE LAPSE OF TIME FOLLOWING INTRAVENOUS INJECTION OF COLLOIDAL LEAD

IDENTIFICATION NUMBER	369	368	366	365	371	370	362	363	372	367
Mg. of Pb injected	12.3	12.3	10.2	12.3	12.3	12.3	12.3	12.3	12.3	12.3
Time interval after injection	25 min.	1 day	3 days		7 days		1 mo.		6 mo.	
Lead in Milligrams Found in Tissues										
Kidneys	0.36	0.39	0.42	0.13	0.07	0.04	0.03	0.00	0.00	0.00
Liver	4.70	2.94	1.50	3.90	1.98	0.90	0.68	0.28	0.00	0.05
Bile			0.00	0.05	trace	0.00	0.00	0.00	0.00	0.00
Spleen	0.24	0.05	0.07	0.05	trace	0.00	0.00	0.00	0.00	0.00
Fat	0.00		0.00	0.00	trace	0.00	trace	0.00	0.00	0.00
Central nervous system	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02
Blood	0.19	*	0.05	0.04	*	*	0.05	trace	0.00	0.00
Muscle	0.32	0.29	0.00	0.07	trace	trace	0.09	0.09	0.04	0.00
Bone	2.16	1.35	1.62	5.60	2.60	4.20	5.20	1.38	1.50	1.74
Lungs			0.09	0.15	0.06	trace	0.00	0.00	trace	0.00
Heart			0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Int. tract and contents	0.81	1.92	1.11	2.16	0.90	1.29	0.10	0.08	0.13	0.32
Skin	†	†	†	†	†	†	0.09	0.15	0.00	0.09
Remainder	0.96	0.96	0.54	1.21	0.76	0.96	2.00	0.99	0.78	0.03
Entire carcass	9.74	7.90	5.40	13.36	6.37	7.42	8.25	2.99	2.46	2.25
Lead in Milligrams per 100 Grams of Tissue										
Kidneys	2.25	2.29	2.21	0.72	0.31	0.27	0.16	0.00	0.00	0.00
Liver	5.87	2.69	2.73	4.70	1.86	1.33	0.78	0.44	0.00	0.05
Bile			0.00	10.00		0.00	0.00	0.00	0.00	0.00
Spleen	8.00	2.50	3.50	5.00		0.00	0.00	0.00	0.00	0.00
Fat	0.00		0.00	0.00			0.00		0.00	0.00
Central nervous system	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Blood	0.54		0.08	0.05			0.05		0.00	0.00
Muscle	0.08	0.07	0.00	0.02	0.00		0.01	0.02	0.01	0.00
Bone	1.22	0.58	1.00	4.79	1.54	2.33	2.48	0.75	0.65	1.05
Lungs			0.10	1.36	0.35		0.00		0.00	0.00
Heart			0.00	0.00		0.00	0.00	0.00	0.00	0.00
Int. tract and contents	0.21	0.33	0.38	0.67	0.29	0.46	0.02	0.02	0.02	0.08
Remainder	0.11	0.12	0.07	0.22	0.10	0.13	0.21	0.10	0.05	0.01–
Relative Concentration of Lead in Tissues—Ratio $\frac{\text{Mg. per 100 Grams Tissue}}{\text{Mg. per 100 Grams Total Carcass}}$										
Kidneys	4.63	6.42	8.53	0.91	0.94	0.62	0.58			
Liver	12.07	7.52	10.50	5.92	5.53	3.11	2.87	4.16		0.79
Bile				12.64						
Spleen	16.45	6.97	13.52	6.32						
Fat										
Central nervous system										
Blood	1.12		0.31	0.06			0.17			
Muscle	0.16	0.19		0.02			0.05	0.14	0.09	
Bone	2.51	1.62	0.40	6.02	4.60	5.48	9.12	7.03	11.21	15.64
Lungs			0.40	1.72	1.06					
Heart										
Int. tract and contents	0.44	0.93	1.45	0.85	0.88	1.07	0.08	0.18	0.40	1.16
Remainder	0.23	0.33	0.25	0.27	0.30	0.31	0.76	0.92	0.78	0.03

*No blood sample available by reason of death of animal.
†Skin and ears discarded.

TABLE

LEAD EXCRETION OF RABBITS FOLLOWING INTRAVENOUS

SUCCESSIVE DAYS EXCRETION	AQUEOUS SOLUTION OF $PbCl_2$									COMMERCIAL
	242	244	255	250	254	249		278		
	12 MG. Pb IN 2 DOSES 1 WK. APART AS INDICATED BY*. KILLED AT END	4 MG. AND 6 MG. Pb 1 WK. APART AS INDICATED BY*. KILLED AT END	12 MG. Pb IN 2 DOSES 1 WK. APART AS INDICATED BY*. KILLED AT END	12 MG. Pb IN 2 DOSES 1 WK. APART LAST DOSE 1 MONTH BEFORE EXCRETORY PERIOD. KILLED AT END	12 MG. Pb IN 2 DOSES 1 WK. APART 6 MO. BEFORE EXCRETORY PERIOD. KILLED AT END	12 MG. AND 6 MG. Pb 3 MO. APART. LAST INJECTION 11 MO BEFORE EXCRETORY PERIOD. KILLED AT END		6 MG. Pb IN 1 DOSE 11 MO. BEFORE EXCRETORY PERIOD. KILLED AT END		
	LEAD IN MG.	LEAD IN MG.	LEAD IN MG.	LEAD IN MG.	LEAD IN MG	LEAD IN MG.		LEAD IN MG.		
	COMBINED FECES URINE	COMBINED FECES URINE	COMBINED FECES URINE	COMBINED FECES URINE	COMBINED FECES URINE	FECES	URINE	FECES	URINE	
2	Lost	(0.61)†								4
1	0.67*	0.44*	0.41*							4
1	0.48	0.39	0.24							4
										(3 No. 359)
1	0.72	0.73	0.19							4
1	0.41	0.67	0.15							4
3	0.58	0.91	0.35							4
1	0.60*	0.75*	0.32*							4
1	0.62	0.95	0.67							4
1	0.27	0.99	0.28							
1	0.25	0.99	0.25							
4	0.17	0.46	0.38							
4	0.12	0.71	0.09							
3 (2 No. 242)	0.27	0.11	0.31							
4		0.27	0.04							
4		0.24	0.09							
4		0.28	0.00							
4 (1 No. 244)		0.47	0.00							
4			0.07	0.32	0.31	0.21	0.03	0.21	0.03	
4 (5 No. 254)			0.31	0.50	0.39	0.11	0.05	0.20	0.02	
4 { 3 No. 250 } { 3 No. 255 }			0.27	0.46	0.17	0.24	0.05	0.23	0.00	
Total excretion	5.16	9.36	4.42	1.28	0.87	0.56	0.13	0.64	0.05	
Lead in carcass in mg.	5.91‡	5.10	3.77‡	3.82	1.72	2.33		3.33		
Average daily excretion: First 4 days	0.57	0.56	0.25							
Average daily excretion: Entire period	0.25	0.27	0.09	0.12	0.07	0.06		0.06		

†Not included in total excretion.

‡Including contents of alimentary tract not shown in table.

The analytical results on the animals which were sacrificed at the end of six months are like those shown after a corresponding interval in Table I.

The analytical data obtained from the study of lead excretion following intravenous administration of lead compounds, are shown in Table VII. The excretory response of the animals treated either with lead chloride or colloidal lead was prompt and definite. The two series of results are not directly comparable, because the urine and feces were not collected separately in the case of the animals treated with lead chloride, but they are of the same general order of magnitude. (For reasons which will appear later, the results on the lead chloride animals must be regarded as merely indicative of a trend which is not necessarily accurate in its details.) In sharp contrast, the lead excretion of the animals treated with colloidal lead orthophosphate showed little or no increase. A slight elevation of the excretory rate in the feces appears to have resulted, if the findings are compared with those obtained on normal rabbits, while the urinary lead, though slightly higher than

VII

ADMINISTRATION OF VARIOUS LEAD COMPOUNDS

PREPARATION OF COLLOIDAL $Pb_3(PO_4)_2$						COMMERCIAL PREPARATION OF COLLOIDAL Pb											
361		359		360		364		362		363		373		372		367	
CONTROL. NO TREATMENT. KILLED AT END OF EXCRETORY PERIOD		15.2 MG. Pb AT START OF EXCRETORY PERIOD. DIED 15TH DAY		15.2 MG. Pb AT START OF EXCRETORY PERIOD. KILLED 32ND DAY		CONTROL. NO TREATMENT		12.3 MG. Pb AT START OF EXCRETORY PERIOD. KILLED 28TH DAY				CONTROL. NO TREATMENT. KILLED AT END OF EXCRETORY PERIOD		12.3 MG. Pb 6 MO. BEFORE EXCRETORY PERIOD. KILLED AT END OF EXCRETORY PERIOD			
LEAD IN MG.		LEAD IN MG.		LEAD IN MG.		LEAD IN MG.		LEAD IN MG.		LEAD IN MG		LEAD IN MG		LEAD IN MG.		LEAD IN MG.	
FECES	URINE	FECES	URINE	FECES	URINE	FECES	URINE	FECES	URINE	FECES	URINE	FECES	URINE	FECES	URINE	FECES	URINE
0.20	0 01	0.48	0.07	0.33	0.00	0.21	0.06	1.66	0.32	3.50	0.60						
0.18	0.00	0.38	0.09	0.39	0.07	0.19	0.04	0.91	0.22	1.50	0.17						
0.16	0.00	0.14	0.00	0.36	0.02	0.26	0.03	0.42	0.21	0.84	0.15						
0.20	0.01			0.36	0.05	0.05	0.03	0.57	0.18	0.47	0.28						
0.20	0.00			0.30	0.02	0.19	0.03	0.34	0.13	0.42	0.12						
0.26	0.01			0.30	0.03	0.16	0.06	0.17	0.00	0.25	0.17	0.19	0.10	0.51	0.06	0.43	0.02
0.19	0 01			0.20	0.04	0.12	0.04	0.18	0.14	0.28	0.13	0.23	0.00	0.30	0.06	0.31	0.03
0.19	0.03			0.30	0.00												
1.58	0.07	1.00	0.16	2.54	0.23	1.18	0.29	4.25	1.20	7.26	1.62	0.42	0.10	0.81	0.12	0.74	0.05
1.30		9.26		6.69		0.32		8.25		2.99		0.40		2.46		2.25	
0.05		0.14		0.08		0.07		0.49		1.02							
0.05		0.09		0.09		0.05		0.19		0.32		0.07		0.12		0.08	

that of the control animal studied at the same time (Rabbit 361), is little above that of normal animals observed on other occasions (e.g., Rabbits 364 and 373).*

These observed facts are in harmony with expectations based on the chemical properties of the several lead compounds, and they are in accord with the result obtained by Bischoff[4] and his associates in their determination of the toxicity of lead orthophosphate, colloidal lead and certain soluble lead salts.

*Fecal lead output cannot be regarded as an expression of the excretory activity of the tissues because of the variability in the lead content of ingested food. Practically all food substances contain small amounts of lead, most of which is unabsorbed. Contrary to general opinion as expressed in experimental procedures, such lead occurs not in traces, but in readily measurable amounts of high variability. For this reason, we must rely mainly upon the urinary lead excretion for the interpretation of these experimental results, although we may use the alimentary lead excretion of control animals as a means of estimating the approximate magnitude of the ingested lead. The recognition of these facts has led us to separate the urine and feces in all our recent animal experiments and to base our conclusions as to excretory processes chiefly on the urinary findings. The work on lead chloride was nearly completed before we became aware of the importance of the lead in food materials.

The findings in the tissues and in the excreta of human subjects who were treated with colloidal lead compounds are essentially like those obtained in the animal experiments. Table VIII gives the distribution of lead in the tissues of a patient treated for carcinoma of the cervix uteri by the intravenous administration of colloidal tri-lead phosphate. The last dose was administered six days before death. A comparison of the distribution of lead in the tissues of this patient with that found in a rabbit sacrificed one week after similar treatment, shows a high degree of correspondence. A large proportion of the lead was found in the liver, and relatively little in the skeleton, despite the time elapsing between the administration of the first two doses of lead and the death of the patient. Obviously the transfer of lead from

TABLE VIII

DISTRIBUTION OF LEAD IN HUMAN TISSUES AFTER INTRAVENOUS ADMINISTRATION OF COMMERCIAL PREPARATION OF COLLOIDAL LEAD PHOSPHATE

PATIENT A. A. WITH CARCINOMA OF CERVIX UTERI, TREATED WITH 68.4 MG. PB AS COLLOIDAL LEAD PHOSPHATE, 7.6 MG. 3/10/31, 15.2 MG. 3/14/31, AND 45.6 MG. 3/31/31. DIED 4/6/31

TISSUE	WEIGHT IN GM.	LEAD FOUND IN MG.	LEAD IN MG. PER 100 GM.	RATIO: MG. PER 100 GM. OF TISSUE / MG. PER 100 GM. OF ENTIRE BODY
Kidneys	280.0	0.08	0.03	0.21
Liver	1220.0	17.00	1.39	9.93
Spleen	88.0	3.28	3.73	26.65
Fat	185.0	0.00	0.00	
Muscle	25.5	0.01	0.04	0.28
Bone (flat)	29.2	0.15	0.51	3.65
Lungs	1100.0	0.41	0.04	0.28
Heart	290.0	0.00	0.00	
Washed int. tract	1292.0	0.08	0.01–	0.04
Pancreas	160.0	0.12	0.08	0.57
Cancer tissue	235.0	0.04	0.02–	0.12
All tissues	4904.7	21.17	0.43*	

*This figure is faulty because of the small amounts of bone and muscle subjected to analysis. Calculating the total lead in the body on the basis of estimates of the total weights of bone and muscle (approximately 8 and 20 kilograms respectively) the total lead approximates 70 mg. Pb. in a body of 50 kilograms of estimated weight. The gross concentration thus becomes approximately 0.14 mg. Pb per 100 grams. The ratio is calculated on this figure.

the liver (and the spleen) into the bones had been taking place slowly. Furthermore, little had been excreted. The calculation of the total lead in the body is somewhat inaccurate because the actual weights of bone and muscle were not available, and the bones which were analyzed (ribs and sternum) may not have been representative of the lead content of the skeleton in general. Despite these considerations there is no doubt of the essential correctness of the picture when the findings and calculations are compared with those which appear in Table IX. The latter table (IX) shows the results obtained in the analysis of the tissues of a patient whose death from carcinoma of the breast occurred seventeen days after a single intravenous injection of colloidal lead. There are certain differences in the distribution of lead in the tissues in the two cases, viz.; the patient treated with colloidal lead shows a higher concentration of lead in the kidneys, muscle, and heart muscle, a lower concentration of lead in the liver, spleen and bones, and a smaller total amount of lead in the combined tissues. Evidently lead has migrated more rapidly and more generally in the body of the patient treated with col-

TABLE IX

DISTRIBUTION OF LEAD IN HUMAN TISSUES AFTER INTRAVENOUS ADMINISTRATION OF COMMERCIAL PREPARATION OF COLLOIDAL LEAD

PATIENT E. J. WITH CARCINOMA OF BREAST, TREATED WITH 82 MG. PB AS COLLOIDAL LEAD IN ONE DOSE. DIED SEVENTEEN DAYS LATER

TISSUE	WEIGHT IN GM.	LEAD FOUND IN MG.	LEAD IN MG. PER 100 GM.	RATIO: MG. PER 100 GM. OF TISSUE / MG. PER 100 GM. OF ENTIRE BODY
Kidneys	330.0	0.96	0.29	3.22
Liver	3820.0	26.08	0.69	7.67
Spleen	233.0	1.35	0.58	6.44
Fat	365.0	0.16	0.04	0.44
Central nervous system	1312.0	2.03	0.15	1.67
Muscle	172.5	0.08	0.05	0.55
Bone	442.0	0.74	0.17	1.89
Lungs	560.0	0.07	0.01	0.11
Heart	302.0	0.30	0.10	1.11
Thyroid	18.5	0.03	0.16	1.78
Suprarenals	19.0	0.02	0.11	1.22
Uterus	70.0	0.02	0.03	0.33
Hypophysis	1.0	0.00	0.00	
Pancreas	58.5	0.16	0.28	3.11
Cancer tissue	445.0	0.35	0.08	0.89
All tissues	8148.5	32.35	0.40*	

*Correction of this figure must be made as in Table VIII. On the basis of total weights of fat, bone and muscle (approximately 11, 9 and 24 kilograms respectively) the total lead in the body was approximately 64 mg. In the body (approximately 70 kilograms) the calculated concentration would be 0.09 mg. Pb per 100 grams. The figures in the last column are based on this calculated concentration.

TABLE X

DISTRIBUTION OF LEAD IN TISSUES OF NORMAL HUMAN ADULT

YOUNG HEALTHY MALE NEGRO, WITH NO HISTORY OF LEAD EXPOSURE, KILLED BY STAB WOUND OF HEART

TISSUE	WEIGHT IN GM.	LEAD FOUND IN MG.	LEAD IN MG. PER 100 GM.	RATIO: MG. PER 100 GM. OF TISSUE / MG. PER 100 GM. OF ENTIRE BODY
Kidneys	300.0	0.21	0.07	0.41
Liver	2073.0	1.75	0.08	0.46
Spleen	173.5	0.00	0.00	
Fat	109.0	0.00	0.00	
Muscle	388.0	0.00	0.00	
Bone (flat)	92.8	1.03	1.11	6.53
Cartilage	10.6	0.00	0.00	
Lungs	786.0	0.00	0.00	
Heart	276.5	0.00	0.00	
Thyroid	41.5	0.00	0.00	
Suprarenals	10.3	0.00	0.00	
Pancreas	106.7	0.04	0.04–	0.23
Prostate	5.8	0.00	0.00	
Bladder	79.3	0.00	0.00	
Portion spinal cord and femoral nerve	17.8	0.00	0.00	
All tissues	4470.2	3.03	0.07*	

*This figure is corrected as in Tables VIII and IX in terms of total weight of bone (12 kilo.) and total weight of the body (80 kilo.). The total lead comes to approximately 135 mg., giving a concentration of 0.17 mg. per 100 gm. of the entire body. The ratios recorded in the last column are calculated on this basis.

loidal lead than in that of the patient treated with colloidal lead phosphate. It should be noted that there was no selective absorption of lead in the tissue of the tumor in either instance.

The types of distribution of lead shown in Tables VIII and IX are different from each other. Yet both are distinctly unlike that which is found when the usual type of lead absorption, over a period of time, has taken place. Undoubtedly, the character of the distribution of lead in human tissues may be found to vary greatly with a number of factors. Nevertheless when a condition of approximate equilibrium with respect to a prolonged and regular lead absorption has been brought about, the end-result is similar to that which is illustrated in Table X. The data presented in the table were obtained from the analysis of the tissues of a healthy young man who died immediately after a stab wound of the heart. His occupational history gave no evidence of recent industrial exposure to lead.

In Table XI the urinary excretion of lead following the injection of colloidal lead phosphate and colloidal lead are compared. Case A. A. showed no appreciable increase in urinary lead output following the administration of 3 doses totaling 68.4 mg. of lead as colloidal lead phosphate. This fact coincides with the data of Table VIII which show that most of the adminis-

TABLE XI

URINARY LEAD EXCRETION OF HUMAN SUBJECTS FOLLOWING INTRAVENOUS ADMINISTRATION OF COLLOIDAL LEAD COMPOUNDS

COLLOIDAL LEAD PHOSPHATE					COLLOIDAL LEAD				
SUCCESSIVE DAYS' EXCRETION	PATIENT A. A. LEAD IN MG.		PATIENT B. L. LEAD IN MG.		SUCCESSIVE DAYS' EXCRETION	PATIENT P. B. LEAD IN MG.		PATIENT H. LEAD IN MG.	
	FOUND	PER LITER	FOUND	PER LITER		FOUND	PER LITER	FOUND	PER LITER
Control					Control				
2 days	0.02	0.02	0.06	0.05	2 days			0.17	0.11
	7.6 mg. Pb as $Pb_3(PO_4)_2$					49.2 mg. Pb		45.1 mg. Pb	
1 day	0.00	0.00	0.00	0.00	3 days	0.70	0.65	0.02	0.01
1 day	0.03	0.02	0.00	0.00	3 days	0.01	0.01	0.43	0.19
1 day	0.02	0.02	0.00	0.00		82 mg. Pb		71.75 mg. Pb	
1 day	0.00	0.00	0.03	0.02	3 days	0.55	0.42	1.44	0.38
	15.2 mg. Pb as $Pb_3(PO_4)_2$				3 days	0.49	0.28	0.74	0.19
3 days	0.07	0.02	0.07	0.07		41 mg. Pb		57.4 mg. Pb	
3 days	0.04	0.01	0.17	0.14	3 days	0.31	0.17	0.93	0.31
3 days	0.00	0.00	0.21	0.16	3 days	0.35	0.26	0.92	0.34
3 days	0.02	0.01–	0.12	0.11	3 days	0.36	0.19	0.64	0.22
3 days	0.01	0.01–	0.08	0.06	3 days	61.5 mg. Pb		0.30	0.07
	45.7 mg. Pb as $Pb_3(PO_4)_2$				3 days	0.50	0.20	0.27	0.22
3 days	0.05	0.02	0.10	0.09	3 days	0.40	0.15		
3 days			0.28	0.22		45.1 mg. Pb			
2 days			0.28	0.23	3 days	0.44	0.25		
3 days			0.31	0.28	3 days	0.40	0.17		
	95 mg. Pb as $Pb_3(PO_4)_2$				3 days	0.38	0.25		
3 days			0.24	0.19	3 days	0.00	0.00		
4 days			0.38	0.28	3 days	0.29	0.10		
2 days			0.41	0.29	3 days	0.42	0.18		
3 days			0.48	0.32	3 days	0.19	0.19		
2 days			0.18	0.24					
3 days			0.34	0.23	3 days	0.27	0.15		
2 days			0.12	0.17	3 days	0.20	0.12		
3 days			0.31	0.24	2 days	0.10	0.05		
4 days			0.57	0.34	3 days	0.17	0.04		
4 days			0.11	0.07	2 days	0.22	0.06		

tered lead was retained in the tissues. Case B. L. on the other hand, showed a doubtful increase in urinary lead excretion after 22.8 mg. of colloidal lead phosphate had been injected, a definite response after a total dosage of 68.4 mg. had been given, and a still further increase after the gross dosage of 163.4 mg. had been reached. Both patients who were treated with colloidal lead showed a quicker and greater elevation of urinary lead excretion than that seen in the patients who received colloidal lead phosphate. The rate of urinary excretion after the administration of colloidal lead phosphate rises slowly but steadily with each additional increment of injected lead, showing little tendency to diminish between doses. The opposite result occurs after the administration of colloidal lead. A rapid rise in the excretory rate is followed by a rapid fall in most instances. However, the rate after the initial drop is usually well above the level shown by untreated or unexposed persons. These facts are in accord with the other evidences that colloidal lead phosphate is relatively inert in the body while colloidal lead is a comparatively active compound.

DISCUSSION

The foregoing experimental observations make it apparent that the relatively insoluble tertiary and secondary phosphates of lead are chemically and physiologically inert as compared to the compounds formed in the blood when soluble salts of lead, and colloidal lead are injected intravenously. This proves that soluble lead salts are not converted quantitatively into inorganic lead phosphates when introduced into the blood stream. The conclusion that lead is carried in the blood in the form of inorganic phosphates, which was arrived at by Aub[20] and his associates, and by Brooks,[21] after experimentation in vitro, is therefore untenable.

The results obtained on animals treated with colloidal lead show that this compound of lead is almost as reactive in the body as the water soluble salts of lead. Thus it seems probable that colloidal lead is rapidly converted into the same compounds which are formed when lead salts are injected intravenously. This conclusion is in agreement with the experimental observations of Bischoff and Blatherwick,[11] on the toxicity of colloidal lead, and on its chemical instability under conditions similar to those which exist in the blood stream.

The usefulness of the foregoing studies to those who are employing lead compounds in the treatment of malignant tumors, will depend chiefly upon a point of view as to the mode of action of lead therapeutically. If a maintained lead stream of low concentration will combat malignant proliferation, then the use of large doses of lead orthophosphate is justified. On the other hand, if the effect of lead upon tumor tissue depends upon the maintenance of a high concentration of chemically reactive lead in the blood or in the tumor, compounds of lead which are less inert than lead phosphate should be employed. The use of such compounds will certainly give rise to danger in that general lead intoxication in the patient may occur simultaneously with the toxic effect upon the tumor cells, despite the fact that the latter may be more susceptible than the normal tissues.

The hope of obtaining selective localization of lead in tumor tissues, through use of the preparations here studied, is small. The spleen and liver, as well as the lungs, may be expected to remove the greater proportion of lead from the circulation, and the originally high concentrations of lead in these tissues will diminish rapidly (except after the use of the relatively inert lead phosphates) through the migration of the lead into the skeleton, and through excretion from the body.

Finally, it needs to be reemphasized that lead compounds are not stored in living tissues in inert form. Lead continues to be excreted from the tissues until there is a balance between that which is being excreted and that which is being absorbed as the result of unavoidable contacts with lead in food materials and elsewhere. Thus it appears that the mechanisms of lead deposition and metabolism in the tissues are not different in principle from those of the common mineral elements of the body.

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LABORATORY METHODS

AN ADAPTATION OF SAND FILTRATION TO THE RAPID CLEARING OF HEAVY BACTERIAL CULTURE MEDIA

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THE appliance pictured and described below was perfected by the author, with the assistance of his laboratory technicians, and has been used over a period of fifteen years with considerable satisfaction.

The appliance consists of (1) a filtering unit, (2) a collecting bottle, and (3) a vacuum (pressure) pump of sufficient power to produce a negative pressure of at least twenty pounds in a collecting jar of 4,000 c.c. capacity.

The filter consists of a one-gallon glass percolator, the lower end of which is calibrated to accommodate a one-fourth inch heavy walled rubber tube, of sufficient length to reach to the collecting bottle. In the bottom of this percolator is placed a perforated porcelain disc, such as is ordinarily used in a chemical laboratory and of a size that fits snugly. Immediately over this is placed a layer of medium coarse glass wool, two inches thick, and upon this five inches of hot, wet, cleaned sand of a size which will pass a No. 60 mesh screen but be retained by a No. 80 mesh screen.

The culture medium to be cleared, such as nutrient agar-agar or gelatin, is poured, while quite hot, directly upon this layer of sand.

The collecting bottle is equipped with a tightly fitting rubber stopper, with two perforations, through each of which is placed a tightly fitting glass tube about three inches long. To one of these two tubes is connected the rubber tube attached to the percolator; to the other is attached a similar heavy walled rubber tube, the free end of which is attached to the negative pressure pump.

After the pump (which may be electric, or water or hand powered) is started and a negative pressure is produced in the collecting bottle, the culture medium is drawn through the filter and to the collecting bottle, at a rather rapid rate and sufficiently clear for all ordinary bacteriologic work. If egg white is added to the medium to be cleared, the fluid delivered is clearer; the surface of the sand filter may become clogged, however, requiring that it be broken by scraping the surface of the sand with some instrument, such as a heavy stirring rod, introduced through the layer of culture medium over the sand; with which the surface is forcibly broken. This may be repeated several times, if necessary, without interfering with the efficiency of the filter.

The filter has the advantage of filtering sufficiently clear for all ordinary bacteriologic work any of the heavy culture media, including those impaired by contact with organic filter substances, such as Huntoon's Hormone Medium. The media are cleared rapidly, with practically no loss, as the filter may be run until all of the medium is drawn through the filter layers.

The filtered culture medium is unchanged chemically, as it comes in contact with no organic substance with the exception of the rubber tube connecting the

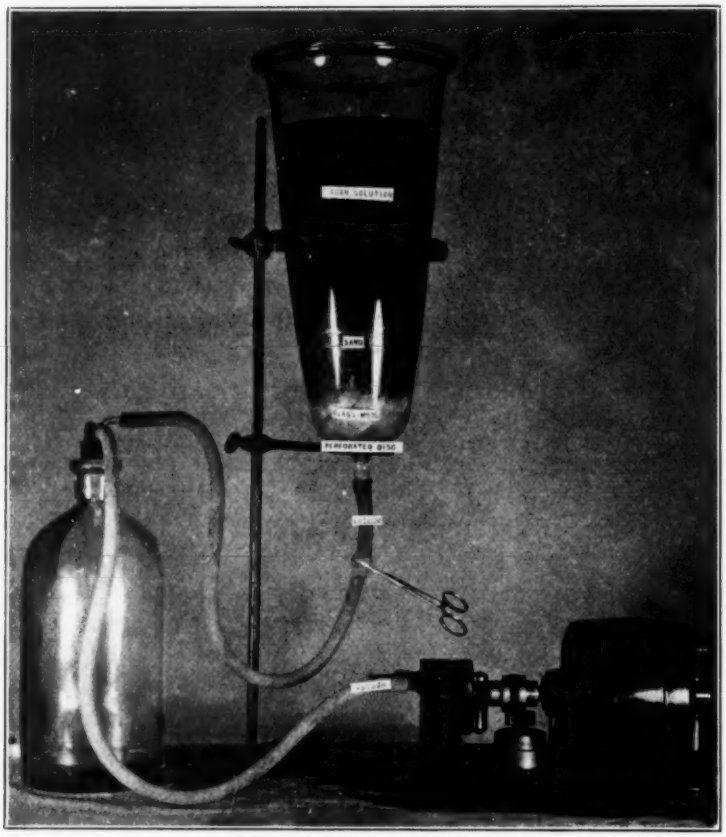

Fig. 1

percolator with the collecting bottle. In our experience this tube has caused no change. If desired, however, this connection may be made with glass tubing.

The filter may be taken down immediately after using, while still hot. The glass wool and porcelain disc may be washed in several changes of boiling water. The sand may likewise be cleaned and used again if desired.

This is the apparatus as used by us in the Bacteriological Laboratory at the Medical College of the State of South Carolina. Its size, and the amounts and sizes of the filtering materials may be changed to suit unusual conditions.

A SIMPLE AND EFFICIENT ELECTRICAL TOOL FOR SEALING MUSEUM JARS*

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THE use of an asphalt cement for sealing museum jars and cells in which pathologic specimens are mounted, while comparatively recent, has proved to be so satisfactory[1, 2] that such a cement is rapidly supplanting those composed of other constituents, especially resins, waxes, and oxides.

Experience with several types of cement, including some of those which are applied cold, indicates that cements containing asphaltum as the principal constituent give the most favorable results and meet nearly all conditions very satisfactorily. When, however, Judah's modification of Kaiserling's solution[3] is used for the final preserving medium there is a tendency for the cement to become liquefied, possibly owing to the liberation of free chloroform from the chloral hydrate which is one of the constituents of the solution. Specimens preserved in solutions without chloral hydrate and sealed with the same cement have not shown a tendency toward liquefaction.

The usefulness of a cement for specimen jars depends on its insolubility, adhesiveness, tenacity, and, in its final physical state, on its plasticity. Cements reach their permanent state by evaporation of the solvent, if used, by oxidation, by a physical change upon cooling, or by a combination of two, or of all three of these processes, depending on the constituents of the original cement. It is obvious that the more quickly the final set is reached the more adapted is the cement for wet tissue mounts.

As asphaltum meets the requirements of a suitable sealing substance, the electrical tool here illustrated and described has been designed for the rapid and efficient application of cements containing it.

The applicator is suitable for sealing practically any type of museum jar and is especially useful for applying asphalt around the cell for thin pathologic mounts as prepared by Davis.[4]

Its advantages are: that it permits quicker and neater work than the usual method, does away with the use of a sand bath for heating, and, to some extent, with disagreeable odors; it is always ready for use, and can be kept in any drawer or receptacle with other tools. Further, one or many containers can be sealed at any time without the usual preliminary preparation or manipulation of the cement. The rectangular jar is prepared in the usual manner; the surface being cleaned first with soap and water, then with absolute alcohol and benzol. It is heated with a flame until hot to the touch, the cement is applied, and the previously heated lid put on and weighted, to insure a thin joint, thus minimizing the amount of surface which can be acted upon by the preserving solution.

*From the Division of Laboratories and Research, New York State Department of Health. Received for publication, December 8, 1932.

Manipulation of the applicator is as follows: The reservoir is filled from the stock container with liquefied cement to a level just above the sluice leading to the point; the apparatus is then tilted back far enough to prevent the cement from flowing out. The current is turned on and the cement allowed to come nearly to the boiling point. The tip of the applicator is then drawn along the top of the jar near the outer edge and the required amount of cement, which can be regulated by the speed at which the applicator is moved, is thus deposited on the surface to be sealed. An excess of cement, or applica-

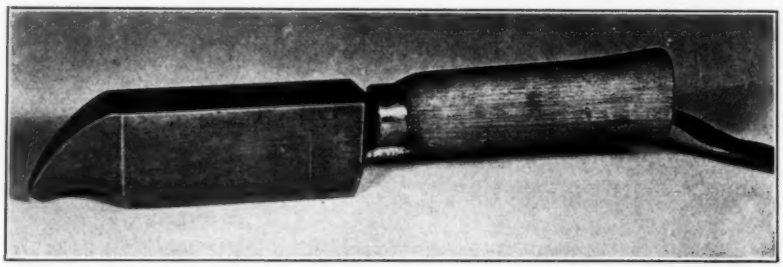

Fig. 1

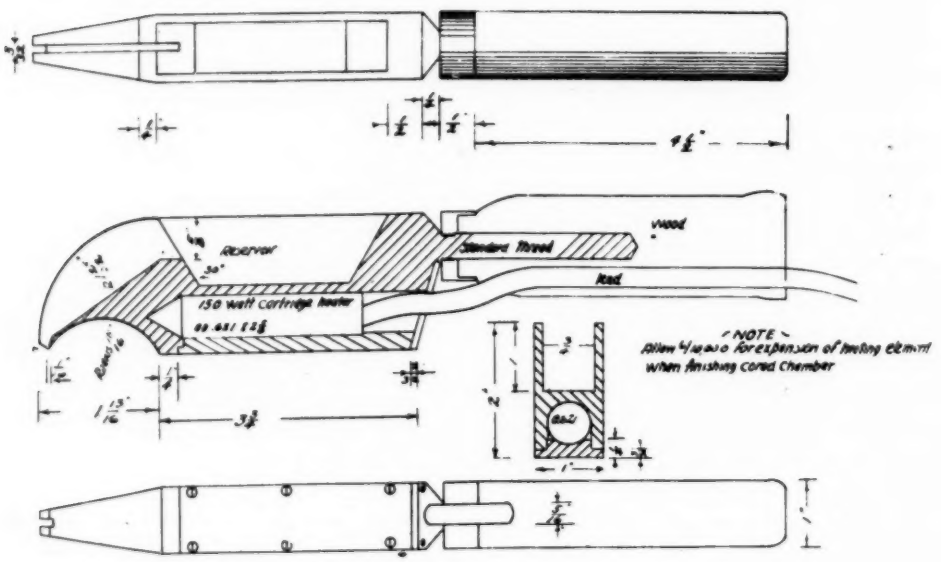

Fig. 2

tion too near the inner edge, may cause superfluous material to be squeezed into the jar when the lid is pressed down. A little practice is needed to gauge the speed and angle at which to operate most satisfactorily.

DESCRIPTION OF THE APPARATUS

The apparatus is 1 by 2 by 11 inches over all and weighs one pound. It is composed of a body, which contains the heating element, a tip or applicating point with a sluice connecting with the reservoir for the cement, and an operating handle of wood (Fig. 1). The applicator is milled from a cored alumi-

nium casting, the dimensions of which are shown on the plan (Fig. 2). The casting was made in a commercial foundry, the shaping, finishing, and electrical work being done in the laboratory machine shop. The heat is supplied by a 150 watt cartridge heating unit placed well forward under the reservoir to insure adequate heating of the point. Such heating units operate on 110 volts, 60 cycle alternating current. As may be noted on the drawing, the applicator base, which is of brass, is removable, in order to facilitate the machine work in the heating chamber. The greatest efficiency in heating is obtained when the unit is in contact with the surrounding metal, but, owing to the difference in the expansion of the metals, a clearance of $^{6}/_{10.000}$ of an inch must be allowed when the chamber is milled. The lead should be asbestos wound and not rubber coated, as there is more heat in the chamber and handle than rubber will withstand.

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A SIMPLE AUTOMATIC SHARPENER FOR MICROTOME KNIVES*

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THERE can be few histologists who have not hoped to find a more efficient method of sharpening microtome knives than the usual hand honing and stropping. The need has been partially supplied by the automatic machines of Fanz,[1] but they are open to two objections. The cost of the apparatus, especially of the machine for larger knives, is prohibitive for many laboratories, and the knife blade is subjected to more wear at the edge of the disc than near the center. The device to be described can be assembled for less than thirty dollars from materials available at any large hardware store, and can easily be adapted for knives of any size.

The principle employed was suggested to me by Dr. Samuel Orton, who built a somewhat similar apparatus, and I most gratefully acknowledge my indebtedness to him. My own additions have been purely mechanical ones.

Sharpening is carried out by resting the edge of the knife against a revolving fiber cylinder (Fig. 1, *A*). The cylinder consists of a "Rockwood" fiber pulley, 9 inches in width and 8 inches in diameter, which has been turned down in a lathe to an absolutely true surface. These pulleys are made for 2 inch shafting, which is too large for convenient use. A ½ inch shaft,

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bearing two 2 inch pulleys as bushings, is inserted in the bore of the large pulley, and is quite strong enough to carry the weight involved. The shaft in turn revolves upon two bronze pillow blocks.

The cylinder is turned by a ¼ h. p. motor by means of a V belt. A motor pulley of 2 inches and a shaft pulley of 8 inches provide a suitable reduction.

If the knife is merely laid on the cylinder, it inevitably becomes grooved by irregularities of the surface. It must be given a slight lateral motion, of about an inch in each direction. This is accomplished by attaching the knife holder to a ½ inch shaft which slides back and forth in pillar bearings, parallel to the cylinder (Fig. 1, *B*). The reciprocating motion is obtained from a crank actuated by a bevel gear lying in the plane of the base of the machine,

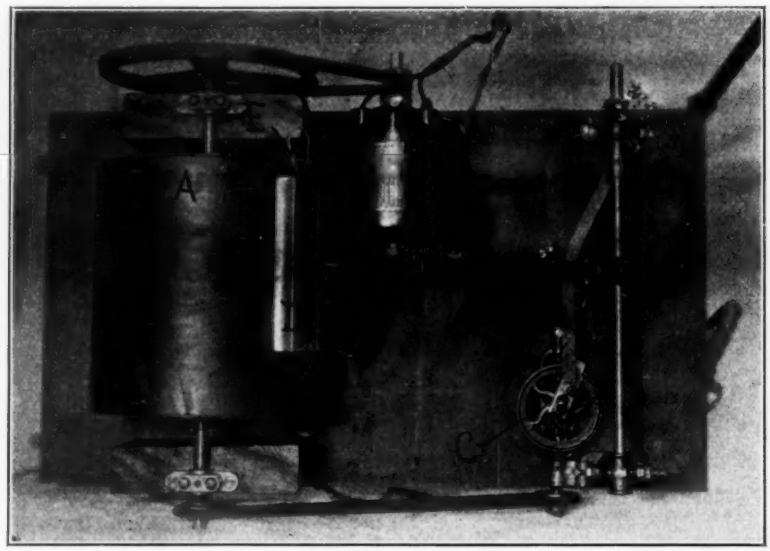

Fig. 1.—Knife sharpener seen from above. *A*, Polished fiber cylinder; *B*, reciprocating shaft; *C*, reciprocating mechanism made from a hand drill; *D*, knife holder; *E*, "filler" fitting inside knife holder to adapt it to knives of different sizes.

and actuated by a small belt and pulley from the cylinder shaft. A cheap hand drill furnishes bevel gears of a convenient size (Fig. 1, *C*).

The knife holder itself is the only part of the apparatus which cannot be purchased ready for use. It consists of a square rod of metal the length and thickness of the back of the largest knife to be sharpened, to which are riveted two flat pieces of spring bronze of equal length and wide enough to hold the knife firmly without striking the cylinder; that is, the edge of the holder comes within about half an inch of the edge of the blade. The bronze strips are bent toward each other so as to hold the knife firmly (Fig. 1, *D*). If smaller knives are to be sharpened also, a small metal "filler" is used, which fits inside the holder and holds the edge of the small knife at a suitable angle to the surface of the cylinder (Fig. 1, *E*). The knife holder swivels on a rod rigidly attached at right angles to the reciprocating shaft. The distance from the reciprocating shaft to the holder is adjustable so that the optimum angle

for the edge of the knife may be found by trial. A weight of about a kilogram should be placed on the rod close to the knife holder to increase the pressure of the blade against the strap (not shown in the illustration).

The only abrasive which is safe to use with this apparatus is jewelers' rouge, made into a thin suspension with castor oil. Nicks no deeper than the honed edge may be removed in ten hours' grinding or less, and no deep grooves are cut. The rouge should be employed as little as possible, however, and carefully washed off with soap and water after use, as a better polish may be obtained with no abrasive at all. A mixture of equal parts of linseed oil and turpentine with a little Japan dryer makes a good dressing and the cylinder gradually acquires an "oil polish." Great care should be taken to keep it clean. It would doubtless be easy to prepare a second cylinder to be used with emery, but this has not proved necessary so far.

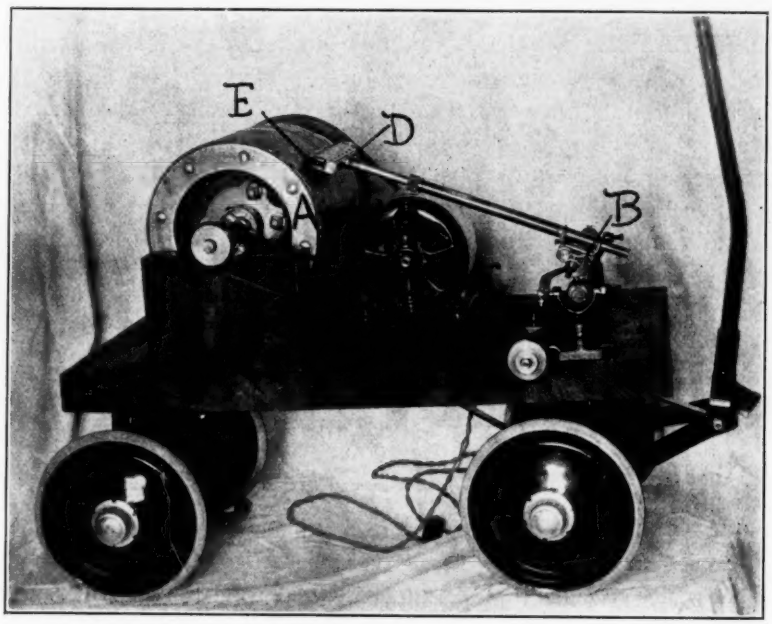

Fig. 2.—Side view of machine. Parts lettered as in Fig. 1.

The angle of the knife should be so adjusted that the polished edge is about 0.5 mm. wide. If it is much wider, a trying "false edge" will form. As the edge is slightly concave, it is sharper than one of similar width produced on a flat hone. The knife should be stropped on one side until only a trifling unevenness is seen under the microscope, then an approximately equal length of time on the other. Wear on the blade is very slow and the machine may safely be left running overnight. When the edge is absolutely straight, the knife is run alternately on one side, then on the other, for a few seconds each during about a minute to remove the burr. A final polish is given by stropping for a minute by hand on a soft strop free from dust or abrasive. The resulting edge is as perfect as can be produced.

The machine may be mounted on the wheels of a toy wagon for easy transportation.

Summary.—A simple automatic sharpener for microtome knives is described, which employs a revolving cylindrical fiber strop. The machine saves most of the labor of sharpening and produces a better edge than can be produced by hand. It is less expensive to build, more compact, less fragile and easier to use than machines now in use, and can be adapted to the largest knives.

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1. Fanz, J. I.: An Automatic Microtome Knife Sharpener and Methods for Grinding and Honing the Knife Satisfactorily, J. LAB. & CLIN. MED. 14: 1194, 1929.

MORPHINE PENTOBARBITAL ANESTHESIA FOR DOG SURGERY*

M. H. SEEVERS, M.D., MADISON, WIS.

A NONVOLATILE anesthetic combination for dogs, which produces good surgical anesthesia and relaxation for a period of from one to two hours and assures postoperative recovery, is a distinct asset to the individual experimenter.

The short-acting barbiturates, pentobarbital and amytal, if used alone, give good anesthesia but recovery cannot be expected for from three to six hours. In addition, since these drugs are not analgesic, undesired restlessness and purposeless movement often characterize the recovery period.

We have been using in this laboratory a combination of morphine and pentobarbital sodium with gratifying results. The anesthetic dosage of pentobarbital sodium for the dog is from 0.025 to 0.035 gm. per kilogram intravenously. If morphine sulphate 0.01 gm. per kilogram and atropine sulphate 0.001 gm. per animal be given subcutaneously one and one-half hours prior to operation, the amount of pentobarbital sodium required is reduced to 0.005 to 0.015 mg. per kilogram, depending on the length of anesthesia required. Obviously, no fixed dosage can be predetermined for any given animal because of individual variability in reaction to the drugs.

When ready for operation, we inject intravenously a 1 per cent solution of sodium pentobarbital slowly, carefully observing respiration and other criteria of anesthesia, particularly the lid reflex. Slow injection (five minutes) permits of maximal control of depth of anesthesia with minimal dosage. The minimal amount of the drug required to produce anesthesia, if given in this manner, will usually last about one to one and one-half hours. Subsequent additions of this barbiturate by the same method of slow injection will extend the period of anesthesia if desired. Precaution against respiratory arrest must be taken by using slow intravenous injection of the drug on a symptomatic basis rather than by rapid administration of a fixed dose.

Recovery is gradual (one to two hours) and is free from excitement. Pharyngeal and laryngeal reflexes return early leaving no concern as to care or ultimate result from anesthetic procedure. We have found this combination highly satisfactory for any physiologic experiment in which the presence of morphine or a barbiturate is not specifically contraindicated.

*From the Department of Pharmacology, University of Wisconsin.
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AN IMPROVED METHOD OF BLOOD CULTURE*

LEONARD S. MANLY, AND CLARENCE C. SAELHOF, M.D., CHICAGO, ILL.

THE following method of making blood cultures has given satisfactory results: A bottle, approximately 250 c.c. in size and containing 30 c.c. of dextrose phosphate broth, is fitted loosely with a two-hole rubber stopper bleeding attachment, the inlet tube of which should extend for about 6 cm. into the bottle, and which should have attached a No. 13 gauge needle, and

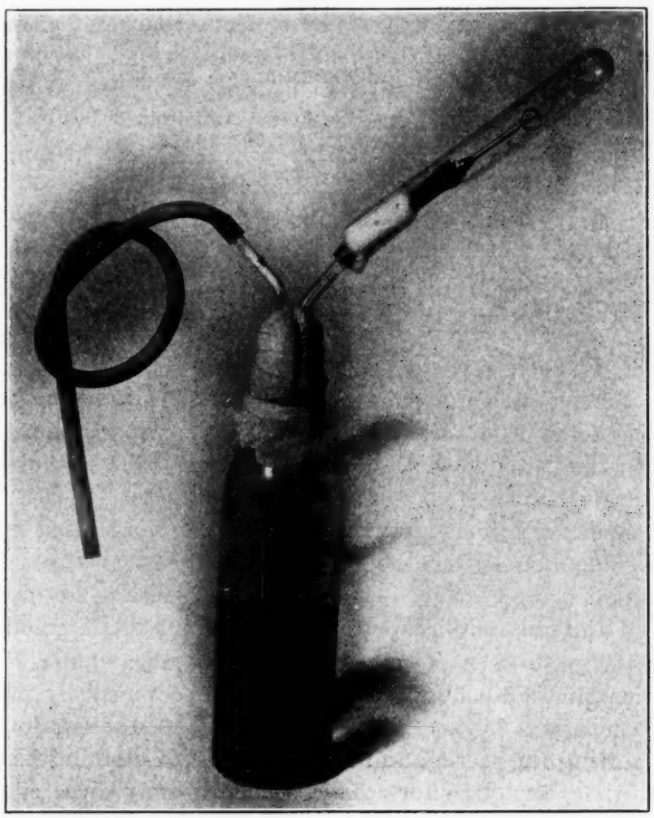

Fig. 1

is sterilized at a pressure of fifteen pounds for thirty minutes. Just before use, as recommended by Hadjopoulos and Burbank,[1] 10 c.c. sterile peptone solution, containing 2 drops of 5 per cent sterile sodium carbonate solution, are added. The rubber stopper is then fitted tightly and tied into position. The apparatus is now ready for use (Fig. 1). Into this bottle approximately 130 c.c. of blood is drawn from a suitable vein. The bottle is left to stand at room temperature for three hours in order to secure a firm adherent clot; it

*From the John McCormick Institute for Infectious Diseases.
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is now incubated at 37° C. and examined carefully each day for the growth of small pinpoint colonies on the edge of the clot or in the surrounding fluid. For identification of eventual growths, streaks on suitable mediums can be made. Using this method positive cultures were obtained in 43 of 67 cases (64 per cent), while the usual routine method yielded positive blood cultures only in 14 cases (21 per cent).

The advantages of the new method are (1) neutralization of natural complement or alexin of blood directly upon withdrawal by the peptone sodium carbonate solution[1]; (2) direct transfer of blood to cultural medium with minimum handling and consequent lessened possibilities of contamination; (3) all gradients of oxygen tension necessary for the growth of different types of bacteria are provided; and (4) the ease of observance of growth.

REFERENCE

1. Hadjopoulos, L. G., and Burbank, R.: Alexin and Antialexic Bodies in Relation to Blood Culture Technic, J. LAB. & CLIN. MED. 15: 662, 1930.

THE PREPARATION AND STAINING OF FROZEN SECTIONS*

A. A. THIBAUDEAU, M.B., BUFFALO, N. Y.

FOR some considerable time there has been much discussion as to the relative value of the various methods used in the preparation of tissues for section and in the methods of sectioning the same. The Pathological Laboratory of the State Institute for the Study of Malignant Disease has for many years been one of the foremost champions of the frozen section method, and as we have many enquiries on this topic, it has seemed desirable to describe in some detail the actual technic in use in our laboratory with some discussion of results obtained and our reasons for the preference of this method.

During the past twenty years tissues from more than 125,000 surgical cases and from almost 1,000 autopsies have been sectioned by the frozen technic in the pathological laboratory of the Institute. Modifications in both the methods of cutting the section and in the technic of staining have been made from time to time, but our efforts have been primarily directed to the preparation of better sections rather than to the reduction of the time necessary to secure the same.

The frozen section method was at first advocated for its rapidity of execution. It was the only method practical for immediate diagnosis of suspicious tissue while a patient was held under anesthetic on the operating table, pending the report of the pathologist. Small portable microtomes were devised and these were especially designed for use in constricted quarters. With such equipment it was obviously difficult to obtain satisfactory or uniform sections. Where speed was the only objective, the quality of the sec-

*From the New York State Institute for the Study of Malignant Disease, Burton T. Simpson, M.D., Director.
Received for publication, January 26, 1933.

tion produced suffered; and this very fact caused many of the older and more experienced pathologists to condemn the method in its entirety.

While these objections were early realized in our laboratory, the desirability to the attending surgeon of prompt reports on biopsy material was also appreciated. Serious attempts were made to so modify and use the frozen section method that examinations could be promptly made without, in any way, sacrificing the quality of the section. At the present time we feel that our routine frozen sections are equal if not superior to sections produced by any other technic.

A prime necessity in the production of good sections is a suitable microtome. We have long since discarded the light, flimsy, portable type of microtome for a more solid instrument. At present we use a large sledge microtome, Spencer No. 860, which we have had modified so that we may use a freezing chamber. Sections produced on this rigid instrument are uniform in thickness

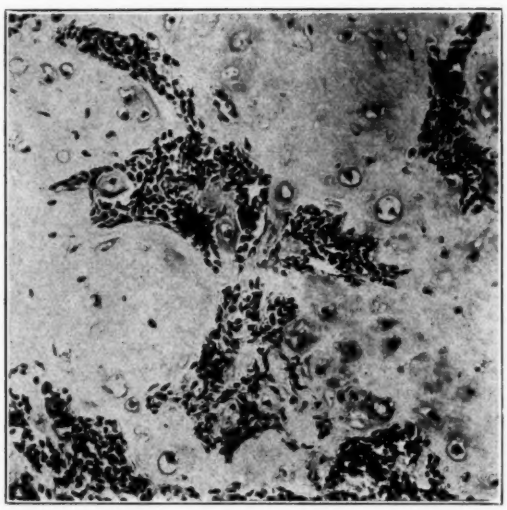

Fig. 1.—Photomicrograph made from a frozen section of a myxochondro-epithelioma. Stained with hematoxylin and eosin.

and can be consistently cut on properly fixed material at from 5 μ to 10 μ. A sharp knife is, of course, essential. We employ 18 cm. and 25 cm. knives which are systematically examined and stropped each day before use. If any serious defect in the cutting edge is noted, the knife is laid aside for thorough sharpening.

FIXATION OF TISSUES

To secure good sections the tissue to be examined must be properly fixed. For the frozen technic, this is best accomplished by the use of 10 per cent formalin on thin slices of tissue. Where used, alcohol or other chemical fixative must be thoroughly removed by washing in water or other suitable solution. In the preparation of material for section the following steps are indicated:

1. Thorough fixation of the gross material preferably in 10 per cent formalin solution.

2. Careful inspection by the pathologist of such material with a view to the selection of suitable blocks for section.

3. Removal of blocks of tissue 1 cm. to 2 cm. square by 2 mm. to 3 mm. thick, and including if possible normal as well as pathologic portions of the tissue.

4. These blocks should be held in 10 per cent formalin until sectioned.

FREEZING OF TISSUES

For the freezing of the tissues carbonic oxide is used exclusively in our laboratory. We have found that tissues must be frozen to just the right consistency. If they are frozen too hard, the sections are apt to be brittle and to break up readily; if too soft the knife scrapes, rather than cuts the tissues and sections are not satisfactory. Various solutions of glucose, dextrin, gelatin, etc., have been used as embedding materials on the freezing stage, but we have

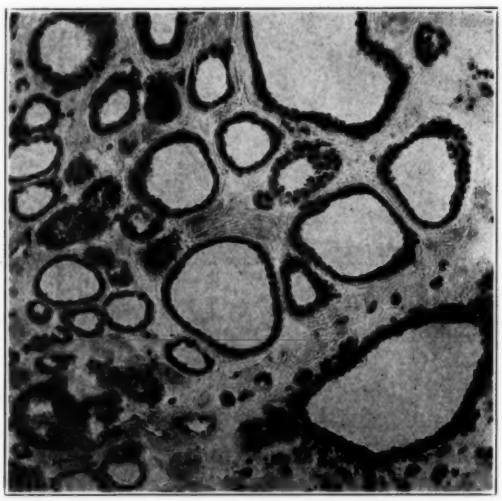

Fig. 2.—Adenoma of thyroid. Photomicrograph made from frozen section. Hematoxylin and eosin stain.

not found any of these to be of great practical value. Water in just sufficient quantity to fix the tissue firmly to the freezing stage has proved most satisfactory and any tissue too friable to give satisfactory sections in this medium alone should be immediately started through the paraffin or celloidin embedding process.

CUTTING OF THE SECTIONS

1. Insert a freshly stropped knife in the holder and systematically tighten all screws holding the knife and the freezing stage of the microtome. We have found that the best sections are secured when the knife is inclined at about 25° to the horizontal and when it sweeps over the block of tissue at an angle of approximately 30°

2. Set the microtome indicator at the proper point, 5 μ, 8 μ, 10 μ.

3. Rinse the block of tissue in distilled water and place in a small drop of water in proper position on the freezing stage.

4. Admit CO_2 to the freezing chamber in short blasts until the water and tissue *start* to freeze. Before the tissue is completely frozen the block is trimmed by bringing the knife over its surface, cutting away excess elevations and leveling the block.

5. When the tissue is well frozen, sections are secured in the usual manner by successive strokes of the knife.

6. Remove sections from the knife with the finger tip or with a wet camel's hair brush to a 10 per cent alcohol solution in a small Petri dish.

STAINING

Sections prepared as above are stained as follows:

1. Deliver sections into a flat Petri dish in the 10 per cent alcohol solution.

2. Transfer whole untorn sections into a shallow staining dish containing a solution of Harris hematoxylin, for from five to fifteen seconds.[a, b]

a. Harris hematoxylin which we have found most satisfactory for routine staining is made up as follows:

Hematoxylin	5 grams.
Alcohol 95%	50 c.c.
Dissolve	
Ammonium Alum	100 grams.
Distilled Water	1,000 c.c.

Boil alum until dissolved: add hematoxylin. Bring whole to a boil. Then add 3 grams mercuric oxide (H_gO) and cool *quickly*. The above is stock solution. For routine staining it should be further diluted as follows:

Hematoxylin (Stock)	300 c.c.
Glycerin	50 c.c.
Glacial acetic acid	12 c.c.

This stain darkens with age and is usually better a few days after preparation. When it becomes too dark, it is discarded and a fresh solution prepared from stock.

b. The placing of the staining dish over a window of the same diameter in the laboratory table and below which a 50 watt electric lamp is placed, gives a satisfactory illumination of the staining dish and allows sections to be handled more easily and more expeditiously.

3. Remove sections with straight section needle to large Petri dish containing fresh distilled water. Leave until all excess stain is washed out. Gentle agitation facilitates this process.

4. Transfer to a second Petri dish containing distilled water to which a few drops of NH_4OH have been added. In this solution the sections at once change from a purple to a blue color.

5. Sections are lifted, in rotation, into dishes containing 70 per cent, 85 per cent, and 95 per cent alcohols respectively, being left a few seconds in each.

6. Counterstain in third saturated solution of eosin in absolute alcohol for five seconds.

7. From the counterstain, sections are transferred for clearing to carbol xylol contained in a fairly large deep staining dish. From this solution they are mounted directly on the slide without the use of a section lifter.

8. Blot sections with filter paper and mount in balsam. This whole process of sectioning, staining and mounting can be carried out in less than five

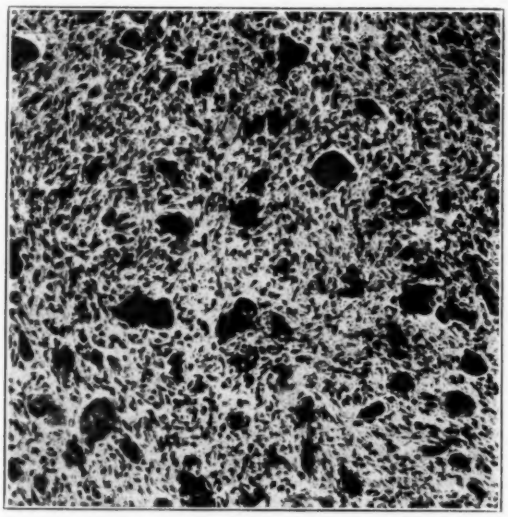

Fig. 3.—Giant cell tumor, epulis. Frozen section. Hematoxylin and eosin stain.

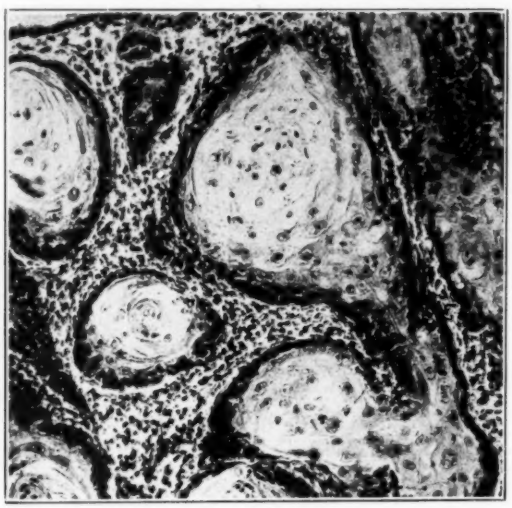

Fig. 4.—Squamous-cell carcinoma of skin. Photomicrograph made this year from frozen section made, stained and mounted in 1913, and in our files since that time. Staining by hematoxylin and eosin.

minutes. We have in some profusion tried out the various rapid methods using fresh unfixed tissues, polychrome stains, special embedding media, etc., but have not found any of them to give the same satisfaction as do fixed tissue sections stained with hematoxylin and eosin. The latter, moreover, give us permanent sections which retain their stain satisfactorily over long periods of time and which are of value in the permanent record of the case.

TABLE DIAGNOSIS

While in our laboratory we are of the opinion that the so-called "Table diagnosis" performed during operation is undoubtedly in many cases of great value to the surgeon, we feel that if this procedure is to be used, accuracy should be the primary consideration. Sufficient time should be allowed the pathologist to thoroughly examine the tissue in the gross and to select and properly fix the pieces of tissue for section. Our method in these cases is to fix small blocks, not more than 2 mm. thick or 1 cm. square, of selected tissue in strong formaldehyde solution which is kept at the boiling point. Fixation is usually satisfactorily accomplished in about fifteen seconds. We then proceed with the regular freezing and staining technic. Reports can usually be made on such tissues in four or five minutes from the time the tissue is handed to the pathologist. We feel strongly that the few additional moments necessary to allow the pathologist to make a careful survey of the tissue presented to him and to prepare and stain a section satisfactory for microscopic examination is, indeed, time well spent. If, on the other hand, the surgeon is unwilling to allow sufficient time for this type of examination, we are of the opinion that the whole procedure might better be abandoned.

Frozen sections when properly made and stained show on examination a brilliancy of staining which is attained by no other method of preparation. Due to a minimum subjection to chemicals, heat, etc., the cells retain their original size and shape to a much greater extent than is the case in paraffin or celloidin embedding. For the same reason also, sections prepared by the frozen technic are much more permanent than those prepared by any other method. Sections in our files for more than twenty years are today as well stained and as satisfactory for diagnosis as when first prepared. In fact, to some extent aging seems to improve these sections. During the past few years many photomicrographs have been made for the illustration of scientific articles from sections made routinely in the laboratory by frozen section technic. That this method is as satisfactory as any other for the purpose of photomicrography is demonstrated by several photomicrographs recently made from sections made during the usual laboratory routine. We also submit a photomicrograph of a section of a pearl forming squamous cell carcinoma which has been in our files for more than twenty years.

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A SIMPLE AUTOPSY STAND FOR MICE*

(Miss) J. M. Hutcheson, Toronto, Canada

UNDER the heading "Laboratory Aids" Dr. Raymond H. Goodale described in this Journal (**18**: 422, 1933) a mouse holder made by nailing four spring-type clothespins to a piece of board. We find that such a holder is admirably suited for guinea pigs but for mice prefer one which is made from the cover of a Petri dish and four "Regal" paper clips. Two of these clips are

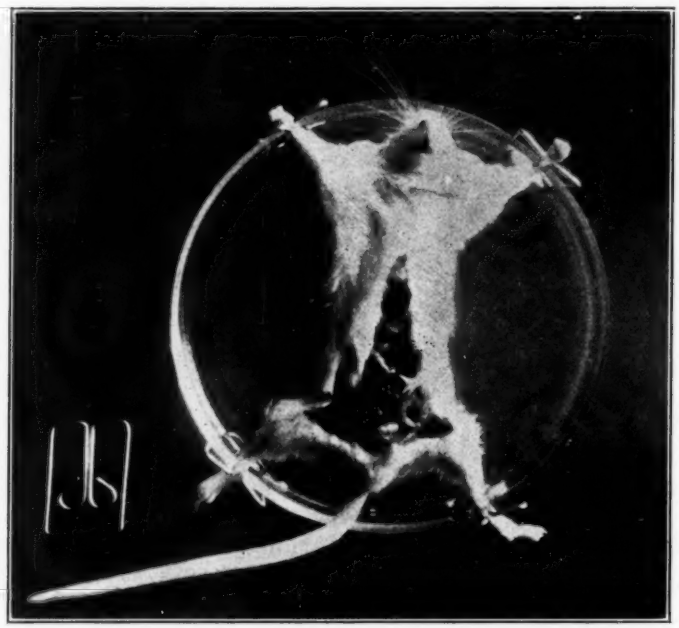

Fig. 1

slipped onto the rim on opposite sides of the dish, and the mouse's legs are drawn through the central slits of the clips. Fig. 1 shows the mouse in position and the type of paper clip which is used.

The advantages of this method are that the holder can be set up and dismantled in a moment; that infective material, such as washings from the mouse's peritoneal cavity, collect in the dish; and that after use the holder and mouse can be dropped into the ordinary basin of disinfecting fluid.

*From the Department of Pathology and Bacteriology, St. Michael's Hospital.
Received for publication, February 24, 1933.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

PNEUMOCOCCUS, Specific Precipitation Test for Standardization of Type I Antipneumococcus Serum, Brown, R. J. Immunol. 25: 149, 1933.

Forty-six Type I antipneumococcus sera were tested for precipitative activity with a solution of the Type I cellular carbohydrate. The results obtained agreed in 91.3 per cent of the cases with the results obtained by mouse protection tests. Eight concentrated antipneumococcus sera were similarly tested and found to agree in seven instances. The results indicate that the precipitation test described provides a titration which appears to be a reliable index of the development of the protective activity in the course of immunization; in the preliminary evaluation of the serum it can thus replace the more complicated protection test which, at present, however, should still be used in the final standardization.

SALMONELLA SUIPESTIFER, Infections in Man, Kuttner, A. G., and Zepp, H. D. J. A. M. A. **101:** 269, 1933.

Four more cases of *Salmonella suipestifer* infection in man are reported: One in an adult and three in children. The adult developed a suipestifer septicemia and cystitis, following a radical antrum operation. Two of the children had arthritis and one of them showed osteomyelitis. The other child developed a suipestifer septicemia accompanied by severe purpura, during the course of bronchopneumonia. No complications such as arthritis, osteomyelitis, cystitis and purpura were observed in the group of cases reported by the authors in 1932. It is of interest that this organism, *Salmonella suipestifer,* Group II, can produce arthritis and osteomyelitis unaccompanied by fever.

Purpura has been observed as a rare complication in paratyphoid B fever. In none of the cases of suipestifer infection in man described in the literature has severe purpura with complete disappearance of platelets been described such as occurred in Case 2. Since purpura is a common symptom of pigs infected with the hog cholera virus, it was thought possible, in view of the interstitial bronchopneumonia found at autopsy, that in this patient *Salmonella suipestifer* might have been associated with the virus of hog cholera. Unfortunately, no experiments to determine the presence of the virus were undertaken. On the other hand, it is entirely possible that the purpuric manifestations were merely the result of a severe infection in a debilitated child and that some virus other than that of hog cholera was responsible for the pathologic picture in the lung. None of the other cases showed any evidence of purpura, and their platelet counts were entirely normal.

Three of the patients live in Baltimore; one lives in the country. In no instance was any association with infected pigs established, and none of the cases had any contact with each other. There was no history of a similar illness in any other member of the family, although the mother of one of the patients agglutinated the organism isolated from her daughter.

The organisms isolated from these four cases all belong to Group II, *Salmonella suipestifer,* although Group I is generally considered the American type, most commonly associated with hog cholera in this country. In recent years, however, Group II has been associated with increasing frequency from infected pigs in the United States.

URINE: Practical Method for Simultaneous Demonstration of Lactose and Glucose in, Kleiner, I. S., and Tauber, H. J. Biol. Chem. **100:** 749, 1933.

To 1 c.c. of urine in a graduated 20 c.c. cylinder, 1 c.c. of a 4 per cent solution of copper sulphate is added, and then enough barium hydroxide solution to make the fluid faintly alkaline to litmus (approximately P_H 7.5 to 8); 5 c.c. is usually sufficient. Enough

water is added to make a total volume of 20 c.c. and the solution is mixed and filtered. If a faint opalescence persists, the results will not be affected and the trace of barium present need not be removed for either of the two determinations to follow. Two cubic centimeters of this filtrate is pipetted into a Folin-Wu sugar tube. Two similar tubes are prepared for standards, one of which contains 0.2 mg. of dextrose in 2 c.c. and the other 0.4 mg. of dextrose in 2 c.c. To each of the three tubes 2 c.c. of monose reagent is added and the tubes are heated in boiling water for eight minutes and cooled for two minutes. This reagent consists of a 1 per cent solution of barium hydroxide, to which is added 5 drops of a 1 per cent solution of sodium bisulphite for each 25 c.c. of reagent just before it is used. Another set is run with the Benedict alkaline copper reagent simultaneously, with the same amounts of filtrate, standards and reagent, boiling for six minutes and cooling for two minutes. To each of the tubes in both sets 2 c.c. of Benedict's color reagent is added and mixed with the contents. The tubes are allowed to stand for two minutes, water is added to the 25 c.c. mark, the contents are mixed thoroughly and colorimetric comparisons are made. If the color is too dark, the determination must be repeated with diluted urine for the copper-barium precipitation. With this procedure the monose method yields dextrose values that correspond closely with values obtained by the Benedict method.

BLOOD, Iron in Human, Sachs, A., Levine, V. E., and Apelsis, A. Arch. Int. Med. 52: 366, 1933.

The average iron content of whole blood, based on analyses of samples from 100 normal men, is 50.01± 2.56 mg. per hundred cubic centimeters. The average for 50 normal women is 42.67± 2.13 mg. per hundred cubic centimeters. The blood of women has a definitely lower iron content than that of men.

Iron in whole blood is almost entirely linked with the hemoglobin molecule. Since the quantity of nonhemoglobinous iron present in the serum and the cellular elements is very small, the error in calculating hemoglobin from whole blood iron is negligible.

On the basis that hemoglobin contains 0.335 per cent of iron we conclude that the average normal blood of men contains 14.93± 0.76 gm. of hemoglobin per hundred cubic centimeters, while the average normal blood of women contains 12.74± 0.66 gm. per hundred cubic centimeters.

By dividing the number of milligrams of iron per hundred cubic centimeters of blood by the first three figures of the red blood cell count, a quotient, which Murphy, Lynch and Howard designated as the iron index, is obtained. We have found the iron index for normal men to be 10.01± 0.65 gm., and for normal women, 9.6± 0.56 gm. of hemoglobin per hundred cubic centimeters.

The iron index is preferable to the color index because of the greater accuracy with which the former may be determined.

The iron color index, based on the ratio of the percentage of iron to the percentage of red blood cells, is also preferable to the older hemoglobin color index. The advantages to be derived from the clinical use of this index lie in its accuracy and in the retention of the convenient +1 or −1 designation of the older hemoglobin color index.

The red and white cell counts, iron, hemoglobin, iron index and iron color index in 63 clinical cases are also included in this report.

TUBERCULOSIS, Comparison of Blood Sedimentation Rate and Vernes Flocculation Test in Pulmonary, James, H. M. Med. J. Australia **11:** 265, 1933.

The work of previous writers was confirmed in that both the blood sedimentation rate determination and the Vernes flocculation test are of considerable value in the control of treatment and prognosis in cases of pulmonary tuberculosis.

Providing all precautions are taken, and with certain recognized exceptions, the figures for both tests were comparable, particularly in those infections which were comparatively early or moderately advanced only. In the very advanced cases it was felt that the Vernes test represented the clinical condition less accurately than the blood sedimentation rate.

Of the two methods of investigation, it must be considered that the blood sedimentation rate estimation is more applicable to everyday clinical work and that there is no information given by the Vernes test which cannot equally be supplied by the blood sedimentation rate.

The advantages of the blood sedimentation rate method may be summarized as follows:

1. Expense: The Vernes test, because of elaborate laboratory methods and material, is a much more expensive test than the sedimentation test.

2. Simplicity: Any medical practitioner can do a blood sedimentation test at the bedside if necessary. Results are immediate and no calculations are necessary. With the Vernes test a certain amount of calculation is necessary, the human element becoming a factor to be considered.

3. Information: The blood sedimentation rate actually gives considerably more information than the Vernes test. In the Vernes test we have an end figure only. With blood sedimentation the rate of fall of the erythrocytes is plotted every five minutes and there is nothing in the Vernes test to correspond to the information received from a study of the curve. The difference between a diagonal and a vertical curve in the sedimentation test is often of paramount importance.

4. Comfort: The one cubic centimeter technic for the blood sedimentation is a very simple matter for the patient and occasions practically no discomfort. For the Vernes test it is necessary to withdraw at least ten cubic centimeters of blood. For the Vernes test also a fast of about fifteen hours is necessary before the test is made.

5. Transport: Blood for the Vernes test must be sent to a center where there are the requisite facilities. The blood sedimentation test may be performed anywhere.

In all the author feels that the blood sedimentation test is of equal value to the Vernes test and is not subject to the same number of fallacies.

Because of its ease of application and greater information supplied, it must be preferred to the more complicated Vernes test.

AGRANULOCYTOSIS, In Childhood, Givan, T. B., and Shapiro, B. Am. J. Dis. Child. 46: 550, 1933.

Twenty-nine cases of agranulocytosis in childhood are reviewed, and an additional case is added.

The instances of agranulocytosis in children with severe antecedent infection must be regarded as a unit.

Finally, a case is presented in detail in which antecedent infection was marked and agranulocytosis was superimposed. Adenine sulphate was used to stimulate the marrow centers, with good results, for with the reappearance of the granular elements, the peripheral blood stream, from which *Staphylococcus aureus hemolyticus* had previously been recovered, became sterile. Of note is the fact that when transfusions were started there was a coincident drop in the total number of white cells and in the percentage of granular cells. One injection of adenine sulphate, however, was sufficient to cause a return of the cellular elements to their previously attained level. The patient was then able to withstand two radical operations on the jaw, and recovery was uneventful.

TRICHOPHYTIN TEST, Report of 350 Cases, Muskatblit, E., and Director, W. Arch. Dermat. & Syph. 27: 739, 1933.

Three hundred and fifty patients were tested intradermally, of whom 300 were clinically mycotic and 50 were not mycotic.

Nonspecific reactions were observed in 4 per cent of nonmycotic cases.

The trichophytin test gave a positive reaction in 72.3 per cent of cases proved mycotic by the laboratory.

Clinical diagnosis was more frequently corroborated by the test than by the laboratory examination; for example, in clinically mycotic cases the test was positive in 60.3 per cent, while laboratory examination was positive in only 39.7 per cent.

Patients with highly inflammatory lesions gave a higher percentage of positive tests; however a positive reaction was found in many cases with limited and slightly inflammatory lesions.

The test was positive in cases in which the infection was due to various fungi of human and animal types, particularly in those caused by *Epidermophyton interdigitale.*

Monilia infections of the skin also gave a positive reaction but less frequently than cases due to filamentous fungi (Trichophyton, Epidermophyton, and Microsporon).

The intradermal test with fungous extracts, while not absolutely specific, is of considerable value in the diagnosis of fungous infections of the skin due to filamentous as well as to yeastlike fungi.

Common fungi such as *Trichophyton violaceum, Microsporon audouini* and *Epidermophyton interdigitale* yield an efficacious preparation when a mixed polyvalent extract is made of freshly isolated strains.

General allergic hypersensitiveness of the skin develops not only in deep inflammatory mycoses but also in many cases of superficial type.

Epidermophyton interdigitale, the most common causative agent of dermatophytosis in the country, is apt to cause a considerable degree of cutaneous allergy.

TULAREMIA: Culture Medium for Rapid Growth of P. Tularensis, Foshay, L. Am. J. Clin. Path. 3: 379, 1933.

Twenty-four grams of "Difco" dehydrated brain-veal agar and 2 gm. of nutrose are dissolved with gentle heat in one liter of distilled water containing 5 gm. NaCl, 0.3 gm. Na_2HPO_4, 0.2 gm. KCl and 0.1 gm. $CaCl_2$. The mixture is divided equally among four 500 c.c. flasks and set in the refrigerator to solidify. After solidification 0.1 gm. of cystine is placed in a small pile on the center of each surface. If care is taken not to stir the cystine around, the medium may be autoclaved safely at fifteen pounds for fifteen minutes without impairing the growth-promoting property of the cystine. Upon removal from the autoclave the flasks are set in a row and each is rotated vigorously in turn until the sedimented cystine is dissolved. This usually requires about ten minutes.

If only 250 c.c. of enriched medium is desired, three flasks are capped and put in cold storage and one is cooled to about 50° C. To this flask is added 10 c.c. of 25 per cent dextrose and 30 c.c. of either ascitic fluid or any sterile serum diluted 1:5. Other flasks are melted and enriched similarly as needed. Slanted tubes are capped as soon as set to prevent water loss. If a liter of finished medium is wanted the base medium may be solidified in one large flask, 0.35 gm. of cystine added, autoclaved, cooled and enriched with 40 c.c. of 25 per cent dextrose and 120 c.c. of ascitic fluid or diluted serum.

P. tularensis will grow rapidly and luxuriantly on the surface but not in the water of syneresis. Growth on slants is best at partial oxygen tension and, because of the tendency of the slants to slump when warmed, they are incubated in a slanting position. Partial oxygen tension is obtained by the tandem arrangement devised by Wherry and Oliver.

The medium is not good for preservation. Cultures will usually not remain viable, as judged by subculturing, for more than eight days. Strains that are being carried along on it are best transferred twice a week. For isolation of *P. tularensis* from infected rodent tissues it is only fairly good, inferior to blood-cystine-dextrose agar and to coagulated egg yolk. Its chief virtues are its capacity to promote rapid and abundant growth, and the short time and ease of making. The base medium can be stored indefinitely at low temperature, and tubes of the enriched medium are good for at least three months if water loss is prevented.

PENTOSURIA, Studies In, Enklewitz, M., and Lasker, M. Am. J. M. Sc. **186:** 539, 1933.

Twelve cases of pentosuria are described, the sugar in all cases being l-xyloketose.

All cases of "glycosuria" in which the concentration of reducing bodies in the urine remains unalterably below 1 per cent should be regarded as possible pentosurias and should be further investigated.

By examining urinary specimens voided before and after the ingestion of a liberal carbohydrate meal, it is often possible to differentiate cases of mild diabetes mellitus, renal diabetes and pentosuria. Fermentation tests, and testing with Bial's reagent will give additional valuable information as to the nature of the urinary sugar.

Xyloketose reduces alkaline copper solution in the cold.

The crystalline structure of racemic xylosazone is described.

The ingestion of amidopyrin markedly increases the output of urinary pentose.

Pentose excretion is uninfluenced by modification of the carbohydrate or protein content of the diet. It is also unchanged by rest, exercise or thyroid extract administered by mouth.

When fed 5 gm. of xyloketose a pentosuric excreted but 0.5 gm. more than usual. The urine of a control taking a similar amount of xyloketose showed no reducing substance. It appears that both pentosurics and normals can utilize or destroy xyloketose when it is administered by mouth.

SEDIMENTATION TEST, In Dermatology, Tulipan, L., and Director, W. Arch. Dermat. & Syph. 27: 759, 1933.

The red cell sedimentation test was performed in one hundred and fifteen patients with conditions representing thirty-five diseases of the skin. The method of Westergren was employed.

The sedimentation rate was usually normal in lupus erythematosus and in erythema multiforme, including the bullous variety.

It was usually increased in tuberculosis of the skin.

It was invariably increased in erythema nodosum and markedly so in the eruption due to phenolphthalein.

It was increased in dermatitis herpetiformis, whereas in other generalized grouped vesicular dermatoses, including dermatophytids, it was normal.

The sedimentation rate in epithelioma varied from normal to a pathologic increase, depending on the extent of the lesion.

It was high in a case of Hodgkin's disease in which there were cutaneous lesions.

It gave borderline readings in mycosis fungoides and erythroderma.

Normal readings were obtained in some of the more common dermatoses.

On account of the increased rate in six cases of pemphigus reported by Pessoa and Ribeira and in two cases reported here the sedimentation test is suggested as a possible aid in differentiating pemphigus from erythema multiforme bullosum.

BILIARY TRACT: Diagnostic Methods and Metabolic Studies in Disease of, Twiss, J. R., and Killian, J. A. Am. J. M. Sc. 186: 418, 1933.

A routine of study for diseases of the biliary tract is presented with findings in 10 control individuals and 30 patients in whom a complete investigation showed no evidence of biliary tract disease.

This investigation has included: History, physical examination, cholecystogram, duodenal drainage, inspection of bile specimens, microscopic examination of biliary sediments, chemical analyses of blood and bile, icterus index determination, van den Bergh reactions and the bacteriologic study of bile.

The findings in both series of patients are presented as standards for comparison with findings in cases of suspected gallbladder or liver disease.

Results indicate that with a normally functioning biliary tract these tests should show:

a. A satisfactory "visualization" of the gallbladder on roentgenographic study.

b. The presence of 30 to 60 ml. of concentrated bile during nonsurgical biliary tract drainage.

c. The absence of pathologic elements on microscopic examination of the biliary sediment in all specimens.

d. Little or no cholesterol in the D_1 and D_2 bile.

e. Bile acids of the concentrated bile fraction 4 to 7 times greater than that of the duodenal bile.

f. The absence of pathogenic organisms which occur repeatedly in bile obtained by sterile duodenal drainages.

g. A blood serum icterus index reading of 4 to 8, a negative direct van den Bergh reaction.

SCARLET FEVER, Heterologous, Trask, J. D., and Blake, F. G. J. A. M. A. **101:** 753, 1933.

The authors have demonstrated, as others before them have done or suggested, that heterogenicity exists among the toxins derived from scarlatinal streptococci. However, the chief interest of this communication lies in the demonstration and study of a new scarlatinal toxin from the blood and pleural exudate of cases of scarlet fever which failed to give a therapeutic response to potent scarlatinal antitoxin.

They do not wish to imply that this is the usual cause of failure in the antitoxin treatment of the disease because they have insufficient data on the incidence of this variety of scarlet fever. However, it is significant that Dr. Wadsworth has already prepared a polyvalent antitoxin which does neutralize the new toxin and the standard Dick toxin.

As regards the clinical features of the disease in three cases, it should be noted that pneumonia developed within the first few days in each case. It is of special interest and, perhaps, not without therapeutic significance, that a large amount of toxin was found in the pleural exudate in one case. The authors believe this to be the first demonstration of scarlatinal toxin in such material. They conclude that:

1. Heterogenicity exists among the toxins found in the blood in scarlet fever.
2. Heterogenicity exists among the scarlatinal antitoxins naturally found in man.
3. Polyvalency is desirable in therapeutic scarlatinal antitoxin.

SCARLET FEVER, Effect of Tonsillectomy on the Development of Immunity to, as Shown by the Dick Test, Kereszturi, C., and Park, W. H. J. A. M. A. **101:** 764, 1933.

From a study of 34 tonsillectomized children as compared to 31 controls it is concluded that from this limited number of cases, it might be said that tonsillectomy has certainly no marked effect on the susceptibility to scarlet fever within six months after it is done, as demonstrated by the changing of a positive Dick test into a negative one.

HYDATIDIFORM MOLE, Differentiation of Pregnancy and, Dabney, M. Y., Flinn, G. G., and Dabney, E. G. J. A. M. A. **101:** 771, 1933.

In view of the emphasis recently laid upon the greatly increased concentration of anterior pituitary sex hormone in the urine in hydatidiform mole and chorionepithelioma, the utilization of this fact as a means of differential diagnosis in the present case report is of definite interest.

In a patient with clinical symptoms suggesting hydatidiform mole, together with a strongly positive Friedman test after the injection of as little as 0.4 c.c. of urine, a dead five and one-half months' fetus was finally expelled.

The case is of further interest from a physiologic standpoint because of the unusually large quantity of anterior pituitary sex hormone demonstrated in the urine shortly before abortion of a nonviable fetus. It is possible that abnormal excretion of this hormone regularly accompanies fetal death without complete detachment of the placenta or that the increased quantity of hormone itself brings about defective fetal development and miscarriage and should be an indication for emptying the uterus in pregnancy, when it is not a sign of hydatidiform mole or chorionepithelioma. If this laboratory finding had been taken as an indication for therapeutic abortion, the present patient, a very anemic woman, would have been spared over a week of bleeding.

Further quantitative hormone studies in cases of threatened abortion and dead fetus are desirable, and the authors suggest revision of existing laboratory criteria for the diagnosis of hydatidiform mole and chorionepithelioma.

LIVER FUNCTION: Galactose Tolerance as a Measure of, Roe, J. H., and Schwartzman, A. S. Am. J. M. Sc. **186:** 425, 1933.

A study of the galactose tolerance of normal subjects and patients with representative types of liver disease has been made by determining the blood and urinary galactose concentration following the ingestion of galactose by mouth. Data are presented showing that following galactose ingestion, the blood galactose concentration is a better indication of liver function than the urinary galactose excretion since it is not influenced to the same extent by a variable renal threshold.

Blood galactose concentrations similar to those of normal subjects were obtained in cases of cirrhosis, metastatic carcinoma, congestive heart failure, toxic and chronic degenerative hepatitis. The urinary galactose excretion in these cases was essentially the same as observed in normal subjects. These studies indicate that the galactose tolerance liver function test may not be expected to give evidence of diagnostic value in chronic liver disease.

Blood galactose values higher than normal were observed in cases of acute catarrhal jaundice. A normal response was obtained in a case of cholelithiasis with marked jaundice. These findings indicate that the galactose tolerance liver function test may be of value in the differential diagnosis of jaundices of infectious and noninfectious origins.

Based upon these studies a new technic for the galactose tolerance liver-function test is proposed. In this test 1 gm. of galactose per kilo of body weight is given by mouth under fasting conditions and the galactose content of samples of blood collected at hourly intervals for three hours following ingestion is determined. Data are presented for the interpretation of findings obtained by this technic.

MONONUCLEOSIS, Infectious: A Diagnostic Test for, Bunnell, W. W. Am. J. M. Sc. **186:** 346, 1933.

The presence of a rather high concentration of heterophil antibodies, demonstrable in the form of sheep cell agglutinins, has been described in the active stages of 4 cases of infectious mononucleosis. Since then tests for heterophil antibodies have been made in over 2,000 cases representing various clinical entities, including additional cases of infectious mononucleosis. The diagnostic value of the test has become increasingly evident.

The serum, from 5 to 8 c.c. of human blood obtained as for a Wassermann test, is utilized for the procedure. For sake of conformity in results it is important to use a standard type of agglutination tube. The author has found a tube 100 mm. in length and 10 mm. in diameter most satisfactory and has used it in all tests, as a slight effect upon the readings is noticed with varying sized tubes. The use of a 2 per cent suspension of sheep cells is likewise desirable, as a heavier suspension interferes with the agglutination phenomenon.

If the serum is inactivated properly and the procedure carried out as described, there should be no hemolysis in the tubes to confuse the readings. (In serum which contains bile the sheep cells are usually hemolyzed and the tests unsatisfactory.)

The author has employed the sheep cell agglutinin test in over 2,000 cases representing 76 clinical conditions. With the exception previously noted, namely, serum disease, he has been unable to demonstrate an appreciable increase of heterophil agglutinins for sheep cells in the sera above the normal dilution of 1 to 8. In 15 cases of infectious mononucleosis he has found a consistent increase in all cases. The titers, ranging from 1 to 64 to 1 to 4,096, apparently depended to a considerable extent upon the stage of the disease at which the serum was obtained and upon the severity of the illness. None of the common conditions manifesting a similar clinical picture such as acute adenitis, tuberculous or syphilitic adenitis, Hodgkin's disease, acute or chronic lymphatic or myelogenous leucemia, aplastic anemia, purpura hemorrhagica, agranulocytic angina or Vincent's angina, has shown an increase in heterophil agglutinin titer.

In view of these findings it seems justifiable to accept the test for heterophil agglutinins for sheep cells as a valuable diagnostic procedure in differentiating infectious mononucleosis from a number of clinical conditions of a far more serious nature.

Cases presenting a suggestive clinical and blood picture whose blood serum shows an agglutination for sheep cells in a dilution of at least 1 to 64 can apparently safely be diagnosed as infectious mononucleosis, and a favorable prognosis given.

PELLAGRA: Review of Cases With Special Reference to Gastric Secretions, Mulholland, H. B., and King, R. L. J. A. M. A. **101**: 576, 1933.

The results of gastric analyses in 107 typical endemic cases of pellagra admitted to the University of Virginia Hospital during the past eighteen years confirm the results found by previous observers in regard to the common occurrence of achlorhydria in this disease, even after due allowance has been made for the presence of this condition in normal persons of different ages. The examination of some patients indicated that this condition might be more or less permanent (true anacidity), but in a few instances some degree of normal function returned. The assumption, however, that the presence of achlorhydria was necessarily correlated with such other manifestations as stomatitis, diarrhea, involvement of the central nervous system and anemia was not borne out by this study. There is no implication that achlorhydria is a primary or uniformly antecedent manifestation of this deficiency disease syndrome.

TUMORS, Carcinoid of the Gastrointestinal Tract, Raiford, T. S. Am. J. Cancer **18**: 803, 1933.

Twenty-nine cases of carcinoid tumors in the gastrointestinal tract have been studied with regard to their clinical and pathological significance. Six of these were malignant and had metastasized to the regional nodes of the liver.

The origin of carcinoids has been a subject of much controversy. It is now generally conceded that they arise from the cells of Kultschitzky or the argentaffine cells occurring in the normal intestinal mucosa. The origin and function of these cells remain a matter of speculation, but they are thought to be related in some way to the chromaffin system.

The pathology of the group is characteristic. Occurring most commonly in the appendix, the carcinoids are yellow, submucous tumors which encroach upon the lumen. Next in frequency in the small intestine, they form small submucous or pedunculated nodules which are usually asymptomatic and are not recognized clinically. Rarely found in the stomach and large intestine, they are of more significance. They are larger, and metastasis occurs in a greater number of cases. They are clinically not unlike adenocarcinomas save for general symptoms of less severity.

The histology of the carcinoids is typified by groups and columns of cells surrounded by a dense stroma of hypertrophied connective tissue and smooth muscle. The cells are small and regular in size, the granular cytoplasm having an affinity for silver. The nuclei are uniform and are heavily dotted with chromatin particles.

The prognosis of the group as a whole is good. Only in the minority of cases which have metastasized is the outlook grave. Even in such an event, if the tumor is recognized clinically before metastasis has become widespread, a good result may be attained through surgical intervention.

MENINGOCOCCI, Variation in Agglutination of Stock Cultures of, Maegrath, B. G. Brit. J. Exper. Med. **14**: 219, 1933.

Great variation in agglutination by "type" sera occurs among single colonies of stock meningococci.

Littledale (Type I) strains show a tendency to overlap into Type III to such an extent that these types appear to be approaching interchangeability.

There is no evidence of a division of group and specific antigens among single colonies derived from stock type cultures.

The so-called Types I and III of the meningococcus are in all probability not fixed types.

MENINGOCOCCI, Rough and Smooth Variants in Stock Cultures of, Maegrath, B. G. Brit. J. Exper. Med. **14**: 227, 1933.

Certain variants of stock strains of meningococci can be detected by the characteristics of their growth on homologous immune-serum agar.

One variant develops haloes in this medium and the other does not.

Investigation of the properties of these variants has indicated that they are of "smooth" and "rough" nature respectively. This is supported by differences in growth in various media, in their fermentation and precipitin reactions and in their effects on guinea pigs.

Agglutination and absorption reactions with sera prepared from the variants do not agree with the results obtained by Enders with his "S" and "R" forms.

These variants are being further investigated.

MENINGITIS, Lactic Acid of Spinal Fluid in, De Sanctis, A. G., Killian, J. A., and Garcia, T. Am. J. Dis. Child. 46: 239, 1933.

Lactic acid is a product of the inflammatory process, and in meningitis the lactic acid of the spinal fluid exceeds that of the blood, and is independent of it. The authors believe that variations in lactic acid of spinal fluid offer more dependable information concerning the progress of a case of meningitis than any other laboratory procedure at our disposal. Its practical use is further enhanced by the fact that within the last few years the technic of its determination has been greatly simplified and its accuracy increased.

SILICOSIS, Silicon Dioxide Content of Lungs in Health and Disease, Mc Nally, W. D. J. A. M. A. 101: 584, 1933.

From experimental observations it is concluded that the normal lung contains 1.13 mg. of silicon dioxide per gram of dried tissue.

A chemical examination of the lungs should be made in every case coming to autopsy, in which there is a history of a dusty occupation.

Any lung containing over 2 mg. of silicon dioxide per gram of dried tissue indicates undue exposure to a dusty atmosphere.

LIVER FUNCTION: Urinary Test as Index of Absorption of Gall Bladder Dye When Given Orally, Rudisill, H., and Hemingway, W. W. J. A. M. A. 101: 593, 1933.

The test, a modification of the Kendall test, has for its purpose the detection of gallbladder dye in the urine after oral administration.

To about 50 c.c. of urine from 1 to 2 gm. of stick sodium hydroxide is added. The mixture is heated moderately, to prevent spattering, in an evaporating dish. When evaporation is nearly complete, exactly 1 gm. of powdered potassium nitrate is sprinkled over the extract, and this mixture is fused at moderate heat until the carbon has all disappeared and the "melt" appears white. When the "melt" has cooled, 20 c.c. of distilled water is added, in which the "melt" readily dissolves. The solution is then filtered and the filtrate is acidified with dilute sulphuric acid until blue litmus is turned red. About 0.5 gm. of sodium nitrite is then added to the filtrate and the mixture is placed in a separatory flask. Five cubic centimeters of chloroform is added and the mixture shaken vigorously. A pink color in the chloroform layer indicates the presence of iodine.

Three arbitrary standards of absorption are described:

1. Good Absorption: This is indicated by a deep purplish pink and is the color obtained as a routine in the normal gallbladder visualization. This color can be reproduced by dissolving 5 mg. of resublimed iodine in 100 c.c. of chloroform.

2. Moderate Absorption: This is indicated by an ordinary pink and can be simulated by 2.5 mg. of resublimed iodine in 100 c.c. of chloroform. This degree has been obtained even with excellent visualization of the gallbladder in patients with poor kidney function.

3. Poor Absorption: This gives a very faint pink that is best seen against a white background, and the standard is made by dissolving 1.25 mg. of resublimed iodine in 100 c.c. of chloroform. When the absorption is poor, the examination should be repeated unless there is normal visualization of the gallbladder.

So far the only source of error encountered has been an erroneous index of the degree of absorption in patients with poor kidney function, as mentioned.

In case of nonvisualization of the gallbladder after the dye has been given by mouth, if the described test is positive there is no question of nonabsorption of the dye, and the gallbladder, liver, or duct system must be abnormal.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor, Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

Discovering Ourselves—A View of the Human Mind and How It Works*

THIS is a volume on normal and abnormal psychology written for the patient. Many such volumes have appeared in the past, most of which have been in greater or less measure infiltrated with ballyhoo. No one but a psychiatrist extremely well versed in human behavior and psychic reactions to suggestion would be qualified to write a popular volume that could be relied upon not to do almost as much harm as good. The problem is still further complicated by the need for a writer who can omit scientific verbiage, and discourse in a manner that will be intelligible to and will continue to hold the interest of the average intelligent layman.

Strecker and Appel have succeeded admirably in fulfilling these requisites. If you have been called an introvert and you want to know what it is you can find a straightforward explanation in this volume, one that will not increase your tendency to introversion but it will enable you to combat logically any morbid exaggerations that you might have in this direction. The extrovert and others with borderline psychiatric states can, indeed, understand themselves after a perusal of this volume.

It is one that can safely be recommended to the intelligent layman.

Histopathology of the Peripheral and Central Nervous Systems†

IT CANNOT be disputed that, while neuropsychiatry is truly a specialty, and an exacting one, neuropsychiatric problems are very often first encountered, although not always recognized as such, in general medical practice. Nor can it be easily gainsaid that for an understanding of the clinical phenomena in this field some grasp of neuropathology is essential.

For these reasons, and because, further, adequate discussions of neuropathology from the viewpoint of the physician at large have not been numerous, the present volume is presented.

It is divided into Part I (four chapters, 56 pages), covering diseases of the peripheral nerves; Part II (seven chapters, 131 pages), in which diseases of the spinal cord are discussed; Part III (nine chapters, 253 pages), covering diseases of the brain; and Part IV (seven chapters, 30 pages), devoted to staining methods used by the author.

It is apparent from these data that the volume is not encyclopedic, nor was it intended to be. On the contrary, there is presented a clear, well-written succinct and yet satisfactorily comprehensive survey of neuropathology which while of interest to workers in this field, should be gratefully received not only by the physician at large but also by the general pathologist. To these it should be very welcome and from them it will doubtless be accorded a hearty reception.

The illustrations, with but few exceptions, are well chosen, excellently reproduced, and informative. The format is acceptable.

This book is well fitted for a place on the doctor's shelf of reference texts.

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*Discovering Ourselves. A View of the Human Mind and How It Works. By Edward A. Strecker, A.M., M.D., and Kenneth E. Appel, Ph.D., M.D. Cloth, pages 306. The Macmillan Company, New York, 1931.

†Histopathology of the Peripheral and Central Nervous Systems. By George B. Hassin, Professor of Neurology, University of Illinois. Cloth, pp. 491, 229 figures. William Wood & Co., Baltimore.

Modern Aspects of Gastroenterology*

THE purpose of this book is to present in an orderly and systematic manner the most valuable of the newer methods of investigation, diagnosis, and treatment in the field of gastroenterology.

While the viewpoint of the author naturally reflects European studies, American literature has not been neglected.

Gastrointestinal, hepatic, and pancreatic diseases are all considered in a satisfactory manner. The price of the volume seems a little high but is probably accounted for in part by the number of illustrations.

Filterable Virus Diseases in Man†

WHILE small, this volume presents a very comprehensive yet succinct survey of the present state of knowledge concerning those diseases at present regarded as caused by filterable viruses.

For those desiring a bird's-eye view of a subject still under investigation and in a state of flux, the book may be accepted as an excellent summary of the investigations in this field to date.

Manual of Veterinary Bacteriology‡

THOSE already familiar with this excellent book will not be surprised to find that it has reached a second edition. To those to whom it comes as a new book, it will be found an authoritative and comprehensive reference work of value, not only to the veterinary, but to the laboratory worker at large. Indeed, considering the number and importance of the diseases which may be transferred from animals to man, this volume may well be read by the physician with profit.

It can be highly recommended as an excellent and standard reference text.

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*Modern Aspects of Gastroenterology. By M. A. Arafa, Medical Assistant to Guy's Hospital, London. Cloth, pp. 374, 32 plates, William Wood & Co., Baltimore.

†Filterable Virus Diseases in Man. By Joseph Fine, M.D., Assistant to the Professor of Public Health, Edinburgh, University. Cloth, pp. 144, William Wood & Co., Baltimore.

‡Manual of Veterinary Bacteriology. By Kelser R. A., Major, Veterinary Corps, U. S. Army. Cloth, pp. 552, 93 figures. The Williams & Wilkins Co., Baltimore.

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EDITORIAL

Hyperinsulinism

ALTHOUGH it has been about twenty years since the development of reliable clinical methods for blood sugar determination, the recognition of the etiology of occasional abnormally low blood sugars has been a development of the postinsulin era. Hypoglycemia due to hyperinsulinism is often but not invariably a constant laboratory finding in the individual case. It is accompanied by certain symptoms which we are accustomed to consider as rather neurotic; nervousness, periods of weakness, abnormal sweating, tachycardia, sinking spells, and occasionally even unconsciousness. One has the feeling that if blood sugar determinations were made more frequently and more carefully on the hyposthenic nervous individual with symptoms such as these, grades of hyperinsulinism would be recognized more frequently. Such a discovery would be a great boon to this large group of sufferers. Unfortunately, however, this has not so far been a very common finding even in the type of individual men-

tioned. John[1] remarks on the apparent relative rareness so far of true hyperinsulinism stating that no one physician sees a score of such patients a year. At the same time it is highly desirable to recognize those cases which one does see.

The logical procedure in the treatment of individuals with symptoms associated with abnormally low blood sugar would appear to be the administration of sugar. This is the procedure which is usually followed, although failures have been reported by this method, probably due to the fact that the feeding of large quantities of carbohydrates stimulates the insulogenic function, thereby creating a vicious circle. The next therapeutic development appears to have been rather more successful and consists in the restriction of carbohydrates to a comfortable minimum with the addition of a reasonable abundance of fats, designed to delay the entrance of carbohydrate into the circulation, thereby avoiding overstimulation of the islets.

Partial resection of the pancreas for hyperinsulinism is a possibility and might appear as logical as subtotal thyroidectomy were it not for the tremendous operative risk and the probability that the remaining tissue would undergo rapid hyperplasia, reproducing the original state. Such operations have been done but the end-results have not yet been observed.

Henry J. John reports an entirely new therapeutic procedure, one which at first glance probably appears quite illogical but which on more detailed study gives promise of being the most logical procedure recommended so far. This briefly is the administration of insulin in hyperinsulinism. He reports its use in only one case but in this case the results were almost spectacular.[1] The single case is reported, with the idea in mind that not many cases of hyperinsulinism are seen, and that it is well therefore to report the results so that others may try the procedure in their occasional cases.

In the particular case reported John places the threshold at which insulin output is stimulated, as 200 mg. per 100 c.c., blood sugar. If the patient's digestive glycemia exceeds 200 the pancreas is stimulated to pour out insulin. If the glycemic level can be kept below 200 the pancreas will not be stimulated. The procedure therefore consists in giving insulin (ten units in this case) *after* meals, and before the peak of the blood sugar curve has been reached. This exogenous insulin starts the curve on its downward trend before it has reached 200. In this way the pancreas is kept at rest. In spite of the fact that the patient already had a hyperinsulinism, the only single time at which she experienced hypoglycemic symptoms during the period of treatment was on one occasion when by mistake she took her insulin before her meal instead of after her meal.

The blood sugar level or threshold below which hypoglycemic symptoms appeared was 70. The administration of a standard meal containing 100 grams of carbohydrates, and without the use of exogenous insulin was followed by a digestion glycemia exceeding 200. This was followed by a fall to 40 mg. per 100 c.c., well below the threshold for hypoglycemic symptoms. The interpretation was that the digestion glycemia of 230 stimulated the pancreas which put out a superabundance of insulin with a resulting hypoglycemia, sufficient to produce symptoms.

On the same diet, with insulin administered after the meal, the peak of the curve was reached at about 160, not high enough to stimulate the pancreatic tissues, and there was no subsequent hypoglycemic trough and no reaction.

During the two months in which the patient was receiving insulin, ten units after meals, she remained entirely symptom-free and gained 17 pounds. The dosage was then gradually reduced and later discontinued. One month later, at the time of the report, clinical improvement had continued without interruption.

Functional hyperinsulinism may result either from overaction of the islands of Langerhans or from some defect in the nerve control mechanism. Organic hyperinsulinism may accompany carcinoma or adenoma of the islands. Neither carbohydrate feeding nor the feeding of fat and carbohydrate has been of benefit in hypoglycemia due to hyperinsulinism from adenoma of the island. John points out that in individuals with hyperinsulinism it is important to differentiate functional disease from neoplasm of the pancreas. Often this can only be finally done following laparotomy. He suggests that on theoretical considerations the response described in his case report would not occur in individuals with organic pathology in the pancreas. In such case, the therapeutic procedure described might be developed into a therapeutic test for differentiation between functional and organic hyperinsulinism.

REFERENCE

John, Henry J.: A Case of Hyperinsulinism Treated with Insulin. Preliminary Report. Endocrinology **17**: 583, 1933.

—*W. T. V.*

To Stimulate Original Research Work on Goiter

The American Association for the Study of Goiter, for the fifth time, offers Three Hundred Dollars ($300.00) as a first award, and two honorable mentions for the best essays based upon original research work on any phase of goiter presented at their annual meeting in Cleveland, Ohio, June 7th, 8th, and 9th, 1934. It is hoped this will stimulate valuable research work, especially in regard to the basic cause of goiter.

Competing manuscripts must be in English, and submitted to the Corresponding Secretary, J. R. Yung, M.D., 670 Cherry St., Terre Haute, Ind., U. S. A., not later than April 1, 1934. Manuscripts received after this date will be held for the next year or returned at the author's request.

The First Award of the Memphis, Tenn., 1933 meeting was given Anne B. Heyman, A.B., M.S., University of Michigan, Ann Arbor, Mich., "The Bacteriology of Goiter and the Production of Thyroid Hyperplasia in Rabbits on a Special Diet."

Honorable mentions were awarded J. Lerman, M.D., and W. T. Salter, M.D., Huntington Memorial Hospital, Boston, Mass., "The Calorigenic Action of Thyroid and Some of Its Active Constituents," Prof. Dr. Stefan Konsuloff, Sofia, Bulgaria, "Experimental Studies on Etiology of Goiter."